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57.—CONIDIA FROM INFECTED BUD-SCALES AND ADJACENT WOOD AS A MAIN SOURCE OF PRIMARY INFECTION WITH THE APPLE SCAB FUNGUS *VENTURIA INAEQUALIS* (COOKE) WINT. By Robert McKay, B.Sc., A.R.C.Sc.I., Department of Plant Pathology, Albert Agricultural College, Glasnevin, Dublin. (Plate 19.) [Communicated by Prof. P. A. Murphy.]

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No. 54.

A METHOD FOR DETERMINING THE RADIUM CONTENT OF ROCKS BY DIRECT ALPHA-RAY COUNTING.

By J. H. J. POOLE, Sc.D.

(Plate 18.)

[Read November 24, 1936. Published separately January 4, 1938.]

Up to the present the radium content of rocks has usually been measured by determining the ionisation current produced by the radon present in the rock. Three main methods have been employed in liberating the radon from the rock. In the earliest method, first employed by the present Lord Rayleigh (1), and Dr. Joly (2), the rock is got into solution by suitable chemical procedure, and then sufficient time is allowed for the equilibrium amount of radon to accumulate in the solution. The radon is then liberated from the solution by boiling, and transferred to an electroscope or ionisation chamber in which the increased ionisation current, due to the presence of the radon, is measured. Mache and Bamberger (3) have developed a modified form of this procedure, in which the radon in the solution is removed by circulating a small volume of gas through the solution, which is simultaneously kept well shaken. After a sufficient time the gas is transferred to an ionisation chamber, where its radon content is estimated as before.

In the second method, due to Joly (4), the radon is liberated by fusing the powdered rock with a mixture of alkali carbonates in an electric furnace at a temperature of about 1000° C. During this process a large amount of CO_2 is evolved, and the radon in the rock powder liberated. The CO_2 is re-absorbed by suitable chemicals, and the radon swept from the apparatus by a stream of air or nitrogen into an electroscope or ionisation chamber, where it is measured. A similar method has been used by Piggot and others (5).

The latest fusion method is due to Evans (6). In this case no chemicals are added to the rock powder, and the radon is liberated by heating in a specially constructed electric vacuum furnace to a temperature of about 2000° C. During the resulting ebullition of the rock, it is claimed that the rock powder is completely de-emanated. Again, the final measurement is made by measuring the ionisation current produced by the radon. Both the fusion methods present the advantages of greater speed and less liability to accidental radio-active contamination, as fewer chemical processes are involved. The danger of

contamination due to added chemicals is completely eliminated in Evans' method, and can be practically eliminated in Joly's furnace method by dissolving the chemicals used and evaporating to dryness immediately before an experiment. By this means the radon, if any, in the chemicals is removed. As the graphite boat used in Evans' method may also contain radon, it also must be heated *in vacuo* to the full working temperature before a run; the rest of the furnace being also de-emanated at the same time.

The main objections to the ionisation current method of measurement are two: (*a*) the natural ionisation current in the ionisation vessel or electroscope, due to α rays from the walls of the chamber, γ rays from surrounding objects, and cosmic rays; and (*b*) the fact that the ionisation vessel must usually be standardised by introducing known amounts of radon into the chamber and observing the resulting increased rate of leak of the electrometer. The trouble with the natural ionisation is that, firstly, it is not constant, due to variations in the local conditions, and, secondly, in magnitude it may be as large as or larger than the current produced by the radon liberated from the rock. Evans has, it is true, largely eliminated the effect of the natural leak by using two balanced ionisation vessels, only one of which contains the radon. The electrometer is arranged to measure the difference between the currents in the two chambers. He has also tackled the uncertainty in radium standards by calculating the theoretical value of the ionisation current produced in a chamber of known dimensions, and comparing this with the current produced by his standards (7). The agreement between the two values was good.

Recent advances in particle counting technique, however, suggest that it should be possible to measure radio-active contents by directly counting the α or γ rays liberated by the rock. W. Vogt (8) has employed a Geiger Muller counter for this purpose, and Evans (9) has also tried a similar method. Large quantities of material are, however, required, and the method only measures the total γ ray activity of the combined uranium, thorium and actinium families. The background count also is large—thus Evans found that it took 300 g. of granite containing $1{\cdot}5 \times 10^{-12}$ g. Ra per g. to double the rate of counting. This renders the method unsuitable for specimens of which only small quantities are available, e.g., oceanic deposits. Alpha particle counting has also been employed by Evans (10), but again the material to be tested was in the solid form and the total radio-activity measured. The rate of counting, too, will necessarily be low, ranging according to Evans from 20 to 200 per hr. per 50 cm.^{2} of rock surface. This method has, however, the great advantage that only α particles will affect the counter, other sources of ionisation being entirely eliminated, except in so far as they may affect the background disturbances. The present paper describes an attempt to combine the advantages of the emanation method with the α particle counting method. It has the advantages of being capable of dealing with smaller quantities of material than either of the other counting methods, combined with a low ratio of stray α particles to those due to the radon from an average rock. Up to the present the method

has only been used for measuring radium contents, but if certain difficulties due to the disturbing effects of water vapour in the counting chamber can be overcome, it should also be applicable to the determination of thorium contents. The main objection to the method is its complication and greater expense compared to the older electroscope or electrometer methods.

The principle of the method is simple. The radon is liberated from the rock specimen by either of the fusion methods or by the solution method. Up to the present only Joly's and Evans' fusion methods have been used. The radon is then transferred to a suitable ionisation vessel, whose isolated electrode is connected to the grid of the first valve of a Wynn Williams type resistance capacity amplifier. The last stage of the amplifier can be connected to a photographically recording oscillograph, or if desired to a simple type of thyraton counter, since the speed of counting will not be high. So far photographic recording has been employed, as it shows at once that the amplifier has been functioning correctly.

Description of Apparatus and Method of Use.

The Amplifier.

The amplifier is a five-stage Wynn Williams type, of which Dr. Wynn Williams kindly provided me with the specification. A very similar amplifier is described by him in Reports on Progress in Physics, vol. 3, 1936. The H.T. current for the last four stages is supplied by Westinghouse rectifiers with suitable smoothing, and the heating current of the last stage pentode is A.C., also mains-supplied. The heating current for the first four stages is supplied from a 100 ampere hour 4 volt lead accumulator. This accumulator is incorporated in the set, and can be charged easily *in situ*. The only modifications I have introduced into the Wynn Williams design are (*a*) grid bias batteries instead of potentiometers on the intermediate stages, and (*b*) an independent H.T. battery for the anode of the first stage with suitable decoupling. Since the voltage used on this stage is only about 30 volts, and the current used very small, the charging necessary for this battery is not very great, and it also can be charged *in situ*. The use of this battery does, I think, slightly improve the functioning of the set. I have found the grid bias batteries to last for at least a year, and there would appear in this design to be less chance of any irregularity in the 4 volt supply affecting the set. It also reduces the current taken from this supply slightly.

As regards the spatial arrangement of the set: the last four stages, the H.T. transformers, rectifiers, and smoothing circuits are all contained in a small double-floor two-wheeled truck. This truck also contains the L.T. battery and the H.T. battery for the first stage. The supply apparatus, i.e., rectifiers, batteries, etc., is mounted on the lower floor of the truck, while the metal box containing the amplifier, valves, etc., is on the upper floor. All parts are carefully screened, the batteries being also screened from the H.T. supply, and the

H.T. transformers are independently screened by being placed in complete tin boxes. The decoupling and coupling circuits for the first stage are contained in the main amplifier box on the upper floor of the truck. The first stage valve is, however, mounted independently on the top of the ionisation vessel, its H.T. and L.T. supplies being conveyed to it through a twin core screened flexible lead. The screening on the lead is connected at its ends to the screening cases of the set, and serves as an earth for the ionisation chamber, and also to carry the return heating current of the first valve. For the first stage a D.E.V. valve is used. Some trials were made with an Osram A. 537 microphone valve, but, although much less sensitive to vibration than the D.E.V., it was found to be not so satisfactory as regards general background "noise." Like most high amplification sets the main trouble experienced was with this background noise, and this difficulty was aggravated by the fact that the set had to be used on the top floor of a three-story laboratory, and any vibration in the building produced noise in the set. The trouble was finally practically eliminated by suspending the ionisation chamber complete with its H.T. supply from the ceiling by means of six long steel springs. The springs supplied by Woolworth for exercisers were found to be excellent for this purpose, and easily obtainable. The main amplifier track was also insulated from vibration due to the flexibility of the wooden floor of the room by supporting it on a set of fifteen strong upholstery springs, horizontal stability being produced by four springs pressing laterally on its ends and sides.

The Ionisation Chamber.

Figure 1 shows the design of the ionisation chamber, the first stage, and the H.T. eliminator for the chamber. Since the voltage required for the H.T. supply is about 1,000, the main high tension supply of the set could not be used. The design of the ionisation vessel could, I think, be further improved so as to diminish the dead space slightly. The chamber is cylindrical in form, the isolated electrode being made of a brass rod 4·75 mm. in diameter. This rod passes through a metal bushing mounted on the lid of the outer brass tube, which is earthed, and acts as a shield. The rod is insulated from its bushing by a quartz sleeve, the joints being made with hard sealing wax. Inside the outer brass tube a smaller steel tube is mounted on glass collars. This tube is closed at the bottom and a vacuum-tight joint made above the upper glass collar with sealing wax. This tube acts as the positive electrode of the chamber, its H.T. supply being brought in by a well-shielded, quartz-insulated lead through the outer earthed brass tube of the vessel. The lid of the chamber is positioned by means of a cylindrical shoulder, which fits down into the outer tube, the vacuum joint being made externally with plasticine. The gases whose radon content is to be measured are admitted through a brass tube and rubber connection mounted on the lid of the chamber. The drawing makes the method of carrying the D.E.V. valve on top of the ionisation vessel sufficiently clear. The valve is

supported on sponge rubber, to which it is attached with a rubber band. Both band and sponge rubber are insulated from the valve with thin sheet mica. The total volume of the chamber is about 1,000 c.c.

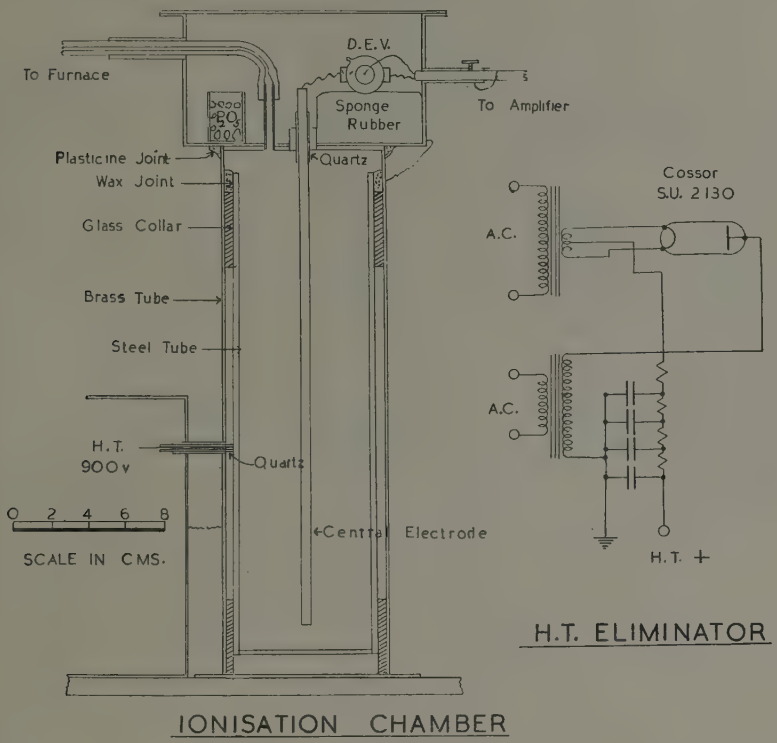

FIG. 1.

The H.T. Eliminator.

This is a simple type, four stages of resistance capacity smoothing being employed. All the resistances are 10 megohm grid leaks. The first three smoothing condensers are 1 microfarad oil-immersed Dubilier paper dielectric condensers, type 951; 6,000 v., D.C. test. The last stage condenser is a 0·1 microfarad mica dielectric condenser, working voltage being 3,000 v., D.C. This component was specially constructed by Dubilier. All stages are independently screened. A half-wave rectifier valve (Cossor S.M. 2130) is used. The H.T. for this valve is obtained from a small transformer, such as is used for hard Cathode Ray Oscillograph tubes. The eliminator is capable of generating a smoothed D.C. voltage of about 3,000 v. Fortunately, it was found that 900 v. was sufficient, since increasing the voltage also increases the back-

ground noise, owing probably to larger irregularities in the leakage currents of the smoothing condensers. The voltage control was effected by a potentiometer in the primary A.C. circuit of the H.T. transformer.

The Oscillograph Equipment.

To record the amplified current impulses produced by the α particles a Muirhead moving iron oscillograph, type 5-B, is employed. This instrument has the advantage of robustness and moderate price, and its natural frequency of about 3,000 c.p.s. seems to be quite sufficient. The oscillograph is coupled to the output pentode of the amplifier through a choke capacity coupling, and its sensitivity can be varied by a shunt resistance across its terminals, which also helps to damp the instrument. The oscillograph record is made on 35 mm. photographic paper in a camera specially constructed to run steadily at low paper speeds. This camera is driven by a small D.C. electric motor through a forty to one worm reduction gear. The final drive from the gear to the camera is a Meccano chain drive, as belt drive was found unsatisfactory, due to slipping. The advantage of the worm gear is that it enables the motor to run at reasonable speeds even for very low paper speeds. The motor is a 100 v. shunt-wound type. It is arranged that the field is always excited by the full voltage, the speed of the motor being varied by changing the voltage across the commutator brushes. The voltage used was generally about 10. The records are made on 200 foot rolls of plate emulsion paper. This paper is slightly more expensive and rather more troublesome to handle than bromide paper, but it greatly simplifies the problem of obtaining sufficient illumination from the small oscillograph mirror. It is arranged that the paper can be cut at any time, and the exposed paper reel removed from the camera for development without interfering with the threading of the unexposed paper, or any part of the drive of the various components. It is necessary, however, to be able to darken the room moderately well during this process. The operation requires only about fifteen seconds,, and the reel can then be placed in a light-tight box for removal to the photographic dark room. The cutting knife in the camera prevents any stray light reaching the unexposed reel during this procedure, and no trouble with fogging was experienced.

The time marks on the paper are made by a light revolving aluminium disk with four equally-spaced slots, which revolves in front of a narrow illuminated slit. The disk is driven from the seconds spindle of a centre seconds synchronous electric clock movement. When the slot in the disk and the slit coincide a narrow vertical beam of light falls on the horizontal slit of the oscillograph, and thus a time mark is produced every fifteen seconds. The method has the advantages of cheapness and simplicity, and could be arranged to give shorter time intervals if desired. Its accuracy, of course, depends entirely on the supply, but from observations on electric clocks the Dublin supply seems to be quite satisfactory in this respect.

Extraction of Radon from Rock Specimens.

As already mentioned, two fusion methods have been tried for liberating the radon from the rock specimen. The Joly method has already been fully described elsewhere (4). The platinum tube furnace used in all the previous determinations was employed, the only modification being that an extra side connection with stop-cock was introduced, so that at the end of the fusion of the rock powder the radon liberated could either be swept into the gold-leaf

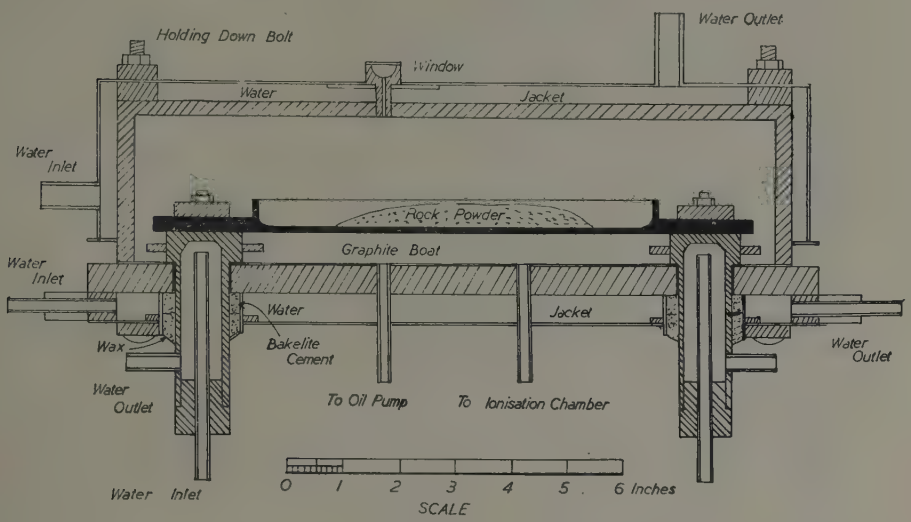

FIG. 2.

electroscope previously used, or into the new α particle counting chamber. The Evans direct fusion method was also employed. As this method is comparatively new, and some modifications have been made in Evans' original design, a brief description of the arrangements employed is given.

A drawing of the furnace is shown in Figure 2. The main differences in the design are:—

(1) Both lid and base plate are cooled with water jackets.

(2) The electrodes are circular in cross-section and cooled through a central channel.

(3) The joint between the lid and the base plate is made with a flat rubber gasket. The lid is firmly held down against the gasket by clamps at each end, which pass completely round the lid and base plate. Short distance pieces inside the water jacket on top of the inner steel lid, and placed vertically under these clamps, ensure that the pressure is directly borne by the inner lid,

and not by the rather lightly-constructed jacket. As the sides of the lower jacket are made of ½″ square brass, a similar precaution is not necessary here.

The electrodes are machined from solid 1¼″ round copper bar. Two brass rings soldered on slightly below the tops of the electrodes serve as anchorages for the bolts which clamp down the graphite boat. The inside of the electrode is drilled out to ¾″ bore to about ⅝″ from the top. The electrode is turned down to 1″ diameter where it passes through the base plate, the shoulder thus produced helping to position the electrode. The method of circulating the cooling water in the electrodes can be easily understood from the drawing. This method of construction has the advantage that there are no soldered joints in the cooling-water system which are exposed to low pressure. The chief difficulty encountered was in making the joint where the electrodes pass through the base plate both vacuum-tight and mechanically strong. The electrodes are insulated from the base plate with a thin rubber washer on top of the plate under the shoulder on the electrode, and a strip of drawing paper round the electrode where it passes through the plate, which is bored to a slightly greater diameter than the electrode. Sealing wax was first tried for making the joint tight, but it was found unsatisfactory, as the life of the joint was short. The final plan adopted, which has proved satisfactory, is shown in the drawing.

A piece of heavy gauge 1½″ brass tubing is soldered onto the lower side of the base plate concentric with the electrode aperture. The tubing is left sufficiently long to project through the lower face of the water jacket, the water joint being made by means of a projecting brass ring near the lower end of the tubing. This ring is also fixed with solder. The upper part of the electrode joint is made with the bakelite sealing compound used for attaching the caps of ordinary electric-light bulbs, the compound being run in when plastic, and then baked to the correct temperature. The compound was kindly supplied by Solus Teoranta, Ireland. This makes a mechanically strong joint, but it may not be vacuum tight. To obviate this difficulty the lower portion of the joint is now filled with black sealing wax. The wax must, of course, be run in when the electrodes, etc., are hot.

The current required for heating the graphite boat is obtained from a specially constructed 10·75 to 1 transformer. Its core is 3 inches square, the primary consists of 258 turns of 8-gauge D.C.C. copper wire. The secondary is laminated, each turn containing three parts of 1⅛″ 18-gauge copper ribbon. The total number of secondary turns is 24. The laminated secondary conductor is insulated with cotton tape wound spirally round the three copper ribbons. The iron core is of the rectangular closed ring type, the windings being wound on plywood wooden boxes. Both primary and secondary are equally distributed between the two vertical sides of the core. The secondary turns are wound outside the primary, from which they are insulated by a layer of dynamo paper and insulating tape. The current is led to the electrodes through laminated conductors, composed of six layers of 1⅛″ 26-gauge copper ribbon. These conductors are bolted and soldered at one end to the secondary winding, and firmly clamped round the bottoms of the furnace electrodes at the other. The

greater flexibility of the lighter gauge copper ribbon renders this operation easier and more effective. The transformer stands immediately behind the furnace, to make these current leads as short as possible.

The transformer was designed for a primary voltage of 346 volts, but owing to the boat resistance being rather lower than was anticipated, a voltage of 200 was found sufficient. This voltage drives a current of about 50 amp. through the primary, corresponding to a power input of nearly 10 kilowatts. The primary current can be controlled by means of a variable liquid resistance. This resistance can, when desired, be entirely cut out with a shorting switch, and as the time in which it is in use is short, the heating of the liquid electrolyte is not a drawback. The power input is approximately measured with an ammeter in the primary circuit. The total cost of raw materials for the transformer did not exceed four pounds, which compares favourably with the commercial price of such a transformer.

Various sizes of graphite boats were tried. The most convenient size was found to be $\frac{5}{8}$″ wide by $\frac{5}{8}$″ deep, the inside of the boat being cut with a vertical milling cutter $\frac{7}{16}$″ in diameter. This boat will hold up to 8 g. of rock powder conveniently, and with a primary current of about 50 amperes a temperature of about 2,000° C. can be reached. The boat is surrounded by a graphite radiation screen, as in Evans' original design. Both boat and furnace are de-emanated immediately before inserting the rock powder by a preliminary heating for at least three minutes at the full working temperature. During this process the whole furnace system is kept continuously exhausted by the rotary single-stage oil pump. To increase the fluidity of the rock melt about 0·7 g. of sodium fluoride was usually added to the 8 g. of rock powder, smaller amounts being used if less rock powder had to be employed. The fluoride was also de-emanated immediately before use by solution and evaporation to dryness in the usual manner.

Figure 3 shows diagrammatically the general design of the furnace and ionisation chamber gas connections. Owing to the fact that the volume of the ionisation chamber cannot be made too large, and that consequently a certain quantity of radon might be left in the furnace at the end of the wash out with CO_2, a simple type of mercury transfer pump is arranged to pump out the furnace into the chamber. The pump is operated by means of the stopcocks 3, 4, and 5 (see drawing); stopcocks 2, 5, and 4 are threeway. The function of stopcock 2 is to connect the ionisation chamber to the platinum tube furnace, when desired.

The mode of procedure in an experiment is as follows. Taps 5 and 4 are set so as to admit air to the mercury bulb. All the other taps except 8 are left open, including the clip on the rubber connection to the ionisation chamber. There is thus direct communication between the latter and the furnace independent of the mercury pump. The rock powder is inserted in the previously de-emanated boat, and the furnace lid replaced. The oil pump is started and the whole system evacuated. During this process the mercury rises in the right hand side of the pump and stands at nearly the barometric height

in the two vertical tubes. To prevent at any stage the motion of the mercury being too rapid, it is necessary to admit a small quantity of air through tap 8. This air is as a precaution drawn from outside the laboratory. The exhaustion attained can be judged from the discharge tube, and the vacuum tightness of the furnace tested by closing tap 7. Finally tap 6 is closed and the furnace heated for about 4 minutes. The internal pressure rises during this process to about half atmospheric. The furnace current is switched off, tap 1 closed, and the furnace exhausted into the ionisation vessel by manipulating taps 3 and 4 in the proper sequence. If at any stage the mercury in the right hand bulb does not rise to the stopcock, when the pressure in the other bulb is atmospheric, the pressure in the latter can be increased by turning tap 5 and gently working the air pump. This was an ordinary cycle pump with a football bladder valve. The actual pressure in the ionisation chamber at any stage can be seen from the

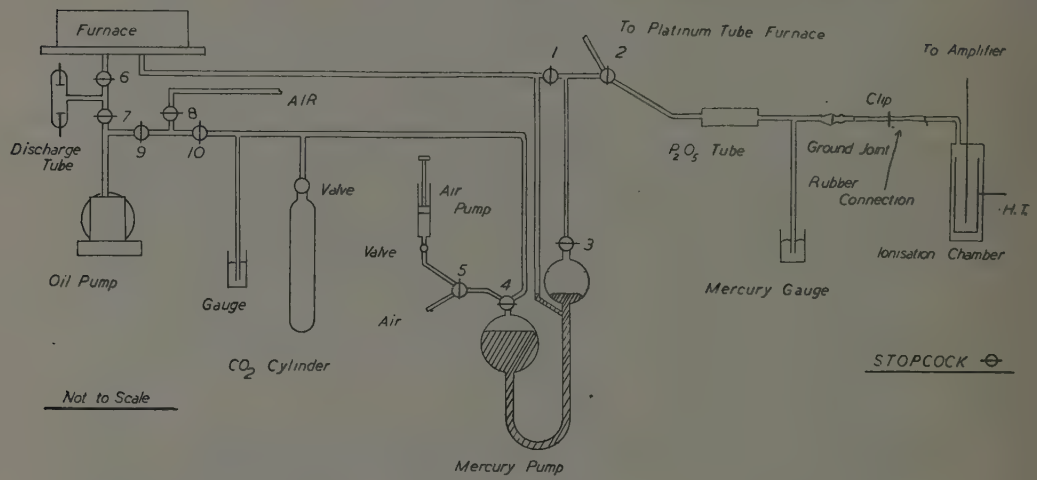

FIG. 3.

mercury gauge connected to it. When the furnace has been exhausted this pressure must still be less than atmospheric. The oil pump is stopped, and the pressure in it raised to atmospheric with CO_2. Taps 7 and 6 are opened gently and the ionisation chamber pressure raised to slightly above air pressure by admitting the necessary CO_2, the final pressure being fixed by the escape of the gas at the bottom of the mercury gauges. The clip on the short rubber connection to the ionisation chamber is finally closed, and the latter completely detached from the furnace system by breaking the ground glass joint. This obviates any danger of mechanical vibration reaching the first valve of the amplifer via this connection. The chamber is now ready for counting. The final count is made about 3 hours after the transference of the radon. During this period the H.T. is applied to the chamber for the whole time, to ensure that the distribution of the active deposit in it will always be the same.

If it is desired to work with the platinum tube furnace, the operation is simpler, since the large evolution of CO_2 sweeps the radon out of the furnace into the soda-lime tube used to absorb the former gas. When the fusion of the rock powder and alkali carbonates mixture is completed, the chamber is exhausted with the oil pump as before. Tap 2 is turned to communicate with the tube furnace with its soda-lime tube, etc., and the radon in the latter swept into the chamber, the extra air required being drawn through the system from an external source. Since in this case the ionisation chamber is full of air and not CO_2 and other gases from the rock, the natural α particle count should also be made with air not CO_2. As a matter of fact, however, the difference found between the two cases was hardly significant.

I have, so far, found it imperative to employ a P_2O_5 drying tube between the ionisation vessel and the furnace. In Evans' method this drying tube was dispensed with, the ionisation chamber insulation being kept dry by P_2O_5 placed in subsidiary chambers inside the main ionisation chamber, but this is difficult to arrange in a smaller vessel. The objection to the P_2O_5 tube is that it may absorb some of the radon, but as so far my results tend to be higher than Evans' it does not appear to be a very serious one. The danger should be much less in any case than with the older platinum tube method where much larger soda-lime tubes are employed for absorbing the CO_2 generated. The reason for the very deleterious action of water vapour in the ionisation chamber is not entirely clear. The first test of the method was made with no drying tube, air being used to complete the filling of the chamber. The resulting background noise in the set rendered α particle counting quite impossible, and this noise showed no sign of abating with time. This seems to show that either large quantities of ions were being continually produced in the vessel, or that the presence of the water vapour was affecting the insulation of the chamber in an irregular fashion and the consequent slight variations in the H.T. voltage were being amplified by the set. The first alternative seems unlikely. It is certain that the original gas from the furnace will be ionised, but under the action of the field in the chamber most of the ions should be quickly removed. It is true that in some cases ionisation may be produced by chemical action, and it is conceivable that the gas mixture in the chamber is not in chemical equilibrium. In the decomposition of the rock powder at high temperatures some water vapour will usually be produced. Some of this water vapour will re-act with the hot graphite boat producing a mixture of hydrogen and carbon monoxide, and when this mixture and air are present in the ionisation chamber, there is a possibility that oxidation might occur. This would have to produce bursts of ionisation to affect the amplifier, since a steady ionisation current has no effect. This hypothesis seems rather improbable, but it was actually found that using CO_2 instead of air did reduce the background noise, but not sufficiently for efficient working. Using CO_2 and a drying tube has, however, cured the complaint, and the main bulk of the effect seems to be due to the action of the water vapour on the electrode insulation. An attempt was made with another

design of chamber to keep this insulation at a higher temperature than the rest of the chamber, but this chamber did not prove a success, mainly I think because the insulation of the positive electrode of the chamber was made of bakelite in this case.

Discussion of Results.

Plate 18 shows two specimens of the type of record obtained, one for 6 g. of gravel from Pike's Peak, Colorado, and the other for the stray α particles in the chamber. The average number of these was about 1·5 per min., which corresponds to 6 g. of rock of radium content $0{\cdot}07 \times 10^{-12}$ g. per g. This shows at once the advantage of the method as regards the increase in the ratio of the effect of the rock to that of local radio-activity, etc. In the electroscope method previously employed, the natural leak of the electroscope was usually about 5·5 divs. per hour, the same leak as would be produced by 6 g. of rock of radium content $0{\cdot}8 \times 10^{-12}$. It is true that as 8 g. of rock was usually employed, the value $0{\cdot}8 \times 10^{-12}$ should be reduced to $0{\cdot}6 \times 10^{-12}$, but still the decrease in the error due to the uncertainty of the natural leak or stray α particle count is large.

The wider vertical marks on the record are the timing marks spaced at 15 second intervals. By improving the optical arrangements these marks could be made narrower, but they do not interfere with the α particle count, as an α particle trace can be seen easily even when it coincides with a timing mark. A run usually lasted for about seven minutes, but for weakly radio-active rocks a longer period may be required.

In order to interpret the records quantitatively it is necessary to know how many α particles per minute will be counted for a known quantity of radon in the chamber. In the succeeding paper I show how we may calculate this figure approximately from the dimensions of the ionisation chamber and the oscillograph records. The figure actually used was, however, determined experimentally by introducing a known amount of radon into the chamber, and counting the α particles directly. The procedure adopted was similar to that previously employed for standardising the electroscope. In 1923 a specimen of borax glass containing a known amount of uraninite per gram was prepared. The uranium content of the uraninite had previously been determined, and hence the uranium and radium contents of the glass are known. This glass was coarsely powdered and has been used in all subsequent standardisations.

The method adopted in this case was first to determine the number of α particles per minute from say 6 g. of a rock powder, then add about 150 mg. of the glass to a fresh 6 g. of the powder, and re-determine the number of α particles per minute. The difference between the two counts is due to the radon liberated from the glass, and as the radium content of the glass is known the number of α particles per minute per 10^{-12} g. of radium in the melt can be calculated. For this purpose a rock of low radium content should be chosen, so that any error due to unequal distribution of the radon in the rock powder may

be reduced. To liberate the radon either fusion with carbonates in the platinum tube furnace or direct fusion in the carbon boat furnace may be used. The former gave a constant of 3·63 particles per minute per 10^{-12} g. of radium, the figure for the latter being 3·58. A mean value of 3·6 has accordingly been adopted, and this figure agrees well with the calculated value. The count is taken about 3 hours after admitting the radon in order to allow radioactive equilibrium to be reached.

In the following table I give the results obtained on some rocks by the new method and the old electroscope method. From this table it can be seen that, whereas the two furnace methods when used with the counter give in most cases concordant values, the electroscope method seems to give higher values, especially for the more radio-active rocks. This discrepancy needs further investigation :—

TABLE.

Specimen.	Radium Content in 10^{-12} g.		
	I	II	III
Granite, Mt. Manitou, Colorado.	2.1	1.93*	1.64
Gravel from top of Pike's Peak.	0.82	0.52†	0.77
Quartz-Mica Schist, from railway tunnel, San Gabriel Canyon, California.	0.58	0.53	0.50
Vesicular Basalt, Chaffee Co., Colorado.	1.7	1.28	1.26
Vesicular Basalt, Idaho Falls, Snake River, Idaho, ...	0.68		0.67
Eclogite, Eppenrenth, Bavaria.	0.15		0.15
Radiolarian Ooze, H.M.S. Challenger, Lat. 7° 25′ S, Long. 152° 15′ W, 2750 fathoms.	1.56	1.13	1.10

Column I gives results using platinum tube furnace and electroscope.
Column II ,, ,, ,, counter.
Column III ,, new direct fusion furnace and counter.

* Only 2 g. of material available.

† This is only a minimum value owing to accidental loss of radon during transference to counting chamber.

In conclusion, I desire to thank Dr. Wynn Williams for his design of the amplifier, and also Dr. E. T. S. Walton for many useful suggestions during the progress of the work.

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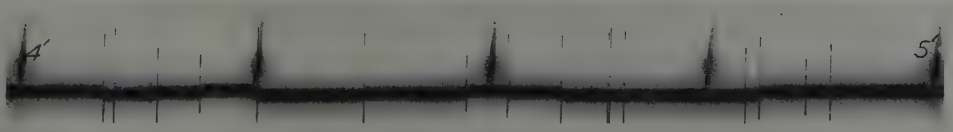
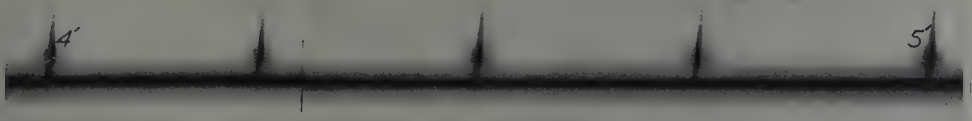


∝ Particles from 6g. Gravel from Top of Pike's Peak Colorado



Stray ∝ Particles

No. 55.

THE THEORETICAL EFFICIENCY OF CYLINDRICAL IONISATION CHAMBERS WHEN USED FOR ESTIMATING RADON BY ALPHA PARTICLE COUNTING.

By J. H. J. POOLE, Sc.D.

[Read November 23, 1937. Published separately January 4, 1938.]

In the preceding paper (1) I have described a method of estimating the radon and consequently the radium content of rock specimens by α particle counting (1). It is plain, however, that the ionisation chamber and amplifier used in this method will not count all the α particles emitted by the radon and its disintegration products, but only some definite fraction of them. This is due to the fact that a certain minimum number of ions must be produced in the chamber before a recognizable kick is produced on the oscillograph record, and consequently α particles whose range in the chamber is too short will escape uncounted. Since at the beginning of their range all α particles produce approximately the same number of ions per mm. of path, we may assume that particles whose path in the chamber is less than a certain value will not be counted. The problem is accordingly to calculate the percentage of these particles.

When radon is introduced into an ionisation chamber, the particles produced will be due to radon, Ra A, and Ra C′. The half life of Ra D being 25 years, Ra F particles may be neglected. If the count is not made till about 3 hours after the introduction of the radon, the number of particles emitted by each radio-active element will be sensibly the same. During this period the radon will have diminished by about 2 %, and this fact must be remembered when comparing the calculated figure with the experimental result.

Figure 1 shows the design of chamber considered. The following notation has been adopted :—

R = radius of chamber (3·57 cm.).
h = height of chamber (26 cm.).
r = minimum range of α particle required to affect oscillograph (1·5 cm.).
N = number of α particles per c.c. per sec. due to radon.

The figures in brackets give the experimental values.

Efficiency for α Rays from Active Deposit.

The outer wall of the chamber is positively charged, and it is consequently assumed that all the Ra A and Ra C′ will be deposited on the central electrode, whereas the radon will be uniformly distributed throughout the volume of the chamber (cf. Evans, 2). It is easily seen that the efficiency of counting for Ra A and Ra C′ will be practically 50 % if the radius of the chamber exceeds the minimum range *r*.

The value of *r* is approximately determined from inspection of the oscillograph records. From these it appears that a number of ions of about $\frac{1}{6}$th that required to produce the maximum deflections in the oscillograph records

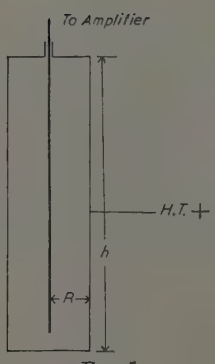


Fig. 1.

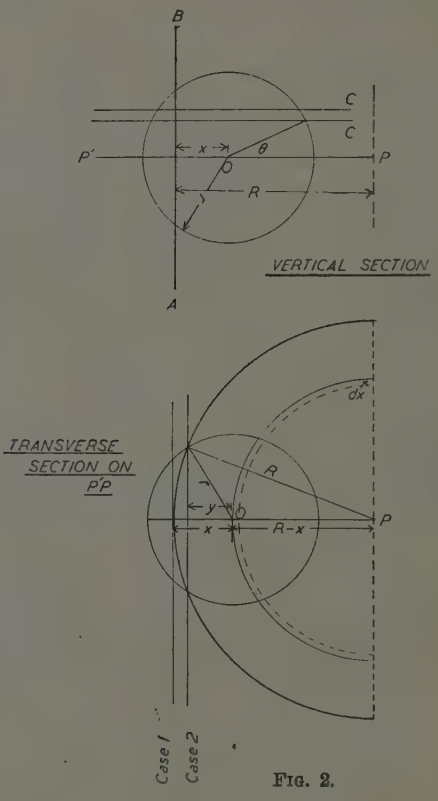


Fig. 2.

would be counted. Now the maximum deflection is produced by a Ra C′ α particle which has completed its range without striking the wall of the chamber. This would occur when the particle was emitted sufficiently obliquely. The

number of ions produced in this case is $2{\cdot}2 \times 10^5$, and, since at the beginning of its path an α particle produces approximately $2{\cdot}5 \times 10^4$ ions per cm.,

$$r = \frac{2{\cdot}2 \times 10^5}{6 \times 2{\cdot}5 \times 10^4} = 1{\cdot}5 \text{ cm. q.p.}$$

Since $R = 3{\cdot}57$ cm., the condition that 50% of the Ra A and Ra C′ particles shall be counted, is satisfied. The fact that a short length at the top and bottom of the central electrode is closer to the ends than 1·5 cm. will slightly reduce this efficiency. This loss can be calculated by a similar method to that later explained for radon, and amounts to about 1 %. Thus the efficiency of counting for Ra A and Ra C′ will be about 49 %.

Efficiency for α Rays from Radon.

In this case the disintegrating atoms will be uniformly distributed throughout the volume of the chamber. The number counted will be reduced owing to α particles striking either the curved sides or plane ends of the chamber when their path in the chamber is less than r. It is more convenient to treat the two sources of loss separately.

A. *Fraction of particles uncounted through striking curved sides of chamber.*

In Figure 2 AB represents a vertical section of the cylindrical wall of the chamber. Let an α particle be emitted at O at a distance x from the wall. With O as centre describe a sphere of radius r. The chance that the α particle is uncounted will be the solid angle subtended at O by the area of this sphere cut off by the cylindrical wall divided by 4π. It can be shown that this chance is equal to

$$\frac{1}{\pi}\int_0^{\cos^{-1}\frac{x}{r}} \cos\theta \,.\, \phi \,.\, d\theta \qquad \text{where} \qquad \phi = \cos^{-1}\frac{R^2 - r^2 \,.\, \cos^2\theta - (R-x)^2}{2\,(R-x)r \,.\, \cos\theta}.$$

This integral cannot be evaluated, and its determination by graphical methods would be very laborious, especially as it would have to be done for a set of values of x ranging from o to r. An approximate method has accordingly been adopted. The cylindrical surface is replaced by a vertical plane. Two cases are considered:

(1) When this plane is tangential to the cylinder. This will give too low a value.

(2) When the plane passes through the points of intersection of cylinder

and sphere which are situated in the horizontal plane containing the atom. This will give too high a value.

The correct value must accordingly lie between these two limits.

Case 1.—If a plane at a perpendicular distance x from the centre of a sphere intercepts it, the solid angle subtended by the intercepted area of the sphere at the centre of the sphere is $2\pi\left(1 - \frac{x}{r}\right)$. The chance of the particle being uncounted is, therefore, $\frac{1}{2}\left(1 - \frac{x}{r}\right)$.

From the transverse section, figure 2, it is seen that the total number of α particles lost is

$$\int_0^r N \cdot 2\pi (R - x)\, h \cdot \frac{1}{2}\left(1 - \frac{x}{r}\right) .dx$$

which is equal to

$$\frac{\pi N h r(3R - r)}{6}.$$

The total number of particles $= N\pi R^2 h$.

Hence fraction lost

$$= \frac{r(3R - r)}{6R^2}.$$

Now $r = 1{\cdot}5$; $R = 3{\cdot}57$.

Thus fraction lost $= 0{\cdot}18$.

Case 2.—It is evident from figure 2 that the chance of an α particle being uncounted is now $\frac{1}{2}\left(1 - \frac{y}{r}\right)$, where

$$y = \frac{R^2 - r^2 - (R - x)^2}{2(R - x)}.$$

Hence total number lost

$$= \pi N h \int_0^r (R - x)\left(1 - \frac{R^2 - r^2 - (R - x)^2}{2(R - x)r}\right) . dx,$$

$$= \frac{\pi N h r(3R + r)}{6}.$$

Fraction lost $= \frac{r(3R + r)}{6R^2}$, $= 0{\cdot}24$ for given values of R and r.

The correct value must accordingly lie between $0{\cdot}18$ and $0{\cdot}24$.

B. *Fraction of particles uncounted through striking plane ends of chamber.*

The number of α particles lost will be

$$2\pi NR^2 \int_0^r \frac{1}{2}\left(1 - \frac{x}{r}\right) . dx = \frac{\pi NR^2 r}{2} .$$

$$\text{Fraction lost} = = \frac{1}{2}\pi NR^2 r \Big/ \pi NR^2 h,$$

$$= \frac{1}{2}\frac{r}{h} = 0{\cdot}03.$$

Thus, collecting the results, the percentage of radon particles counted will be, in Case 1, 79, and in Case 2, 73. Both these figures are slightly too low, due to the fact that some of the α particles which strike the ends have also been counted as striking the sides. This error will be small as $\frac{r}{h}$ is small.

TOTAL EFFICIENCY.

We are now in a position to calculate the total efficiency of the chamber. The results are best shown in tabular form. Let 100 α particles per second be emitted by each of the three disintegrating elements.

Element.	Particles. Case 1.	Counted. Case 2.
Radon	79	73.
Ra A	49	49.
Ra C′	49	49.
Total efficiency	59 %	57 %.

We will not be very far out if we take the calculated efficiency of the chamber as 58 %. It is more convenient to express this figure as the number of α particles per min. per 10^{-12} curie of radon in the chamber. The number of α particles per sec. per g. of radium, free from other radio-active elements, may be taken as $3{\cdot}7 \times 10^{10}$, or 2·22 per min. per 10^{-12} g. There are three radio-active elements in equilibrium emitting α particles in the chamber. Hence 6·66 α particles will be emitted per min. per 10^{-12} curie of radon. Taking our figure of 58 % as the efficiency, 3·86 α particles per min. should be counted per 10^{-12} curie of radon in equilibrium.

As described in the paper already quoted, this figure can be determined experimentally by admitting known small quantities of radon into the chamber. The mean figure thus obtained is 3·60 per min. per 10^{-12} curie of radon

originally admitted into the chamber. To compare this figure with the calculated value it must be increased by 2 % to allow for the decay of the radon during the three hours during which equilibrium is being obtained. The final experimental figure is accordingly 3·67 per min. per 10^{-12} curie.

The agreement with the calculated value is quite good.

The experimental value was determined before any attempt at calculation had been made, and the only real uncertainty in the calculated value is in the estimation of the value of r, the minimum path necessary. The actual value chosen was also taken before the calculation, so there is no question of it being fitted intentionally to the experimentally determined efficiency of the chamber. A small error is introduced, due to some radon α particles having been counted as lost twice—once through striking the walls imagined as continued outside the chamber, and once by striking the ends. It is easily seen, however, that this effect will be small compared with that due to the uncertainty in r.

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No. 56.

THE PREPARATION, PROPERTIES, AND MODE OF OCCURRENCE OF LAMINARIN.

By VINCENT C. BARRY, M.Sc.,
University College, Galway.

[Read January 25. Published separately February 8, 1938.]

Laminarin, which has been the subject of investigations by several authors, is a polysaccharide occurring plentifully in the fronds of marine algae during the autumn months. It was first described by Schmiedeberg (1), who obtained it from Laminariae. It has since been studied by Krefting and Torup (2), Kylin (3, 4, 5), Gruzewska (6, 7), and Colin and Ricard (8, 9). By extraction of dry *L. digitata* with baryta water and acidulation of the extract with acetic acid, Krefting and Torup (2) got a deposit of a material closely resembling the laminarin of Schmiedeberg. It differed from the latter, however, in being insoluble in cold water. Kylin (4), who examined several varieties of marine algae, describes a laminarin soluble in cold water and a closely related body, present according to him only in *L. Cloustoni*, which separates from cold water. By progressive addition of alcohol to aqueous solutions of both varieties he obtained fractions which differed in molecular size and rotatory power. The specific rotatory power of these modifications varied from $-8°$ to $-32°$, and molecular weight determinations by cryoscopic methods showed a decrease of molecular size from about 3000 to 900. The small rotation was associated with the bigger molecules. Kylin thus asserts that laminarin consists of a mixture of closely related polysaccharides which can be fractionated in aqueous solution by precipitation with increasing addition of alcohol. Mme. Gruzewska (7) has made a very complete study of the physico-chemical properties. She describes it as a colloid, whose aqueous solutions possess a granular appearance under the ultra-microscope. The grains are of different sizes and only the small ones show Brownian movement. On allowing the aqueous solution to stand the grains form clusters and lose their Brownian movement. This aggregation of the particles leads to spontaneous deposition of the laminarin. The spontaneous precipitation is accelerated by the presence of acids and oxygen, and is retarded by alkali. She believes that this phenomenon, which is shared by starch and inulin, is due to polymerization. This author also suggests that the different modifications of Kylin could be explained with reference to the degree to which spontaneous aggregation had taken place when the aqueous solution was treated with alcohol. Also the ultra-microscope shows the existence of particles of very different sizes in the aqueous solution.

Colin and Ricard (9) isolated laminarin by a rather complicated and troublesome process. The laminariae were diffused with water and impurities removed by addition of baryta. The concentrated extract was treated with excess alcohol, and the laminarin freed from mannitol by repeated treatments with 85 per cent. alcohol, and finally purified by repeated solution and precipitation by alcohol. These authors attempted to fractionate the laminarin by successive extractions with alcohol of different strengths. They found that the laminarin dissolved for the most part in 70 per cent. alcohol, leaving a small amount which dissolved in 65 per cent. alcohol. These two fractions differed somewhat in rotatory power. Nevertheless these authors regard the conclusion that laminarin is a mixture of different polysaccharides as unjustified by the experimental evidence.

All these investigators studied the hydrolysis of laminarin by acids. Glucose was the only product of hydrolysis, and no other sugar could be isolated. Kylin (4) hydrolysed the laminarin with dilute sulphuric acid, and although he claims to have obtained a complete conversion to glucose, the figures given by him correspond only to somewhat less than 90 per cent. conversion to glucose. Colin and Ricard remark that the glucose determined in their hydrolysate was somewhat low.

A survey of the literature of laminarin thus showed some points in reference to this body on which complete agreement had not been reached:—(1) Is laminarin a definite individual substance or a mixture of closely related polysaccharides differing from one another in degree of polymerization? (2) Is laminarin composed exclusively of glucose? The present investigation was undertaken with a view to clearing up these points and to extend the study of the properties and mode of occurrence of laminarin.

Some years ago the present author, in an endeavour to obtain organic bases from seaweed, allowed fresh *L. digitata* fronds to stand with very dilute hydrochloric acid at ordinary temperatures. After a few days it was noticed that the acid had become milky, and a white powdery deposit settled slowly on the fronds. This substance proved to be identical with the laminarin of Krefting and Torup. The powder was shaken back into suspension, and the milky liquid decanted and allowed to stand for a few days when most of the laminarin had settled. The separation was completed on the centrifuge. Purification was effected by dissolving the laminarin in warm water and allowing it to deposit slowly from the filtered solution. Repetition of this treatment gave a product almost ash-free. The laminarin prepared in this simple manner is very pure. When dried, it forms a dazzling white powder nearly insoluble in cold water, dissolving in warm water at 50° C. to a water-clear non-viscid solution. This slightly reduces Fehling's solution. It gives no coloration with iodine, and is not precipitated by basic lead acetate. As has been previously described by Mme. Gruzewska (7), the aqueous solution undergoes spontaneous precipitation on standing at ordinary temperatures. A 1 per cent. solution becomes milky in about 1 hour, and then deposits spherites which form characteristic flakes

at the bottom of the vessel. The deposition is nearly complete in 24 hours. In the presence of hydrochloric acid a deposit is formed in about 1½ hours. The same solution, however, to which a small amount of dilute sodium hydroxide solution has been added, remains clear for 12 days. Dilute sulphuric, nitric, or acetic acid can also be used in contact with the weed for the preparation of laminarin. Separation of the laminarin takes place more rapidly with hydrochloric acid than with nitric or sulphuric acid, and much more rapidly than with acetic acid. In the case of the mineral acids a milkiness appears in 4–6 hours. With acetic acid a milkiness is evident after two days' standing in contact with the weed. Dilute hydrogen peroxide also deposits laminarin after three days' standing in contact with the weed. 260 g. of dried *L. digitata* fronds, collected on September 9th, 1937, gave 18·5 g. of pure laminarin.

Separation of Laminarin into Fractions.—If, as suggested by Kylin, laminarin consists of a mixture of polysaccharides differing from one another in degree of polymerization, it was thought likely that the successive deposits from an aqueous solution would differ from one another in specific rotatory power and molecular complexity. An aqueous solution containing about 6 g. of laminarin per 100 c.c. was prepared and allowed to stand at ordinary temperatures. Spontaneous deposition of the laminarin took place, and the liquid was decanted from the deposits formed on five successive days. The $\left[\alpha\right]_D$ of each of the five fractions was measured and found to be different in every case. There was, however, no uniform increase or decrease in the rotatory power. It was found on arranging the solutions in increasing order of concentrations that the rotatory power decreased uniformly. It appeared thus that the differences in rotatory power of the fractions of laminarin were due to differences in concentration of laminarin in the solutions employed. Table 1 shows the specific rotatory power and concentration of solutions of successive deposits from an aqueous solution of laminarin.

Table 1.

Successive Deposits.	$\left[\alpha\right]_D^{10°}$	Concentration in g. per 100 c.c.
1st Day	−9·85°	2·186
2nd „	−11·26°	1·336
3rd „	−10·96°	1·600
4th „	−14·12°	0·310
5th „	−13·24°	0·453

Increase in Specific Rotatory Power with Dilution.—A series of determinations of rotatory power of solutions of different concentrations was accordingly made. The figures given in Table 2 show the increase in laevorotatory power with decreasing concentration of laminarin. It appears very probable that increase in concentration causes an increase in the size of the molecule of laminarin. This may be due to polymerization or to a physical aggregation of the particles. If due to the former the molecular weight,

determined from iodine consumption in alkaline solution according to the method of Willstätter and Schudel (10), should also increase with concentration.

TABLE 2.

Concentration in g. per 100 c.c.	$[\alpha]_D^{10°}$	Concentration in g. per 100 c.c.	$[\alpha]_D^{10°}$
5·436	−6·81°	1·596	−10·96°
3·624	−8·14°	1·433	−11·16°
2·718	−8·55°	0·994	−11·82°
2·536	−9·31°	0·717	−12·91°
2·450	−10·00°	0·453	−13·24°
2·304	−10·20°	0·359	−13·95°
1·812	−10·65°	0·239	−14·64°

Table 3 shows a series of results which, while not agreeing very well with one another, indicate that the chemical molecule does not change with the dilution. Taking the average molecular weight 5884, the number of glucose units in the molecule is approximately 36. $[(C_6H_{10}O_5)_n + H_2O = 5884.]$

TABLE 3.

Concentration in g. per 100 c.c.	Molecular Weight.	Concentration in g. per 100 c.c.	Molecular Weight.
0·273	5810	1·148	6042
0·392	6533	1·300	5964
0·406	5042	1·590	6281
0·407	5985	2·026	6535
0·555	6318	2·176	6080
0·717	5253	2·304	5201
0·715	5720	2·408	6320
0·764	5876	3·089	5192

Hydrolysis.—The hydrolysis of laminarin was first effected by heating with 5 per cent. sulphuric acid on a boiling water-bath until there was no further change in the rotation. The glucose estimated polarimetrically never exceeded 90 per cent. of the yield calculated from the equation

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6.$$

It was considered possible that the laminarin might contain a small amount of a laevo-rotatory sugar like fucose which occurs to a considerable extent in sea-weed. The neutral hydrolysate failed to yield any osazone but that of glucose. When the neutralized hydrolysate was fermented with yeast, the glucose was completely destroyed in the course of a few days, and an optically inactive liquid was obtained. That the laminarin is built exclusively on a glucose basis was established finally by using hydrochloric acid in place of sulphuric acid as hydrolytic agent. 99 per cent. was found polarimetrically in the hydrolysate.

Mode of Occurrence of Laminarin.—Laminarin has been obtained, by the

method outlined above, from the fronds of *L. digitata, L. saccharina,* and *Saccorhiza bulbosa.* Kylin isolated it from *L. Cloustoni,* and Colin and Ricard from *L. flexicaulis.* It appears to behave as a reserve food material, being present to a considerable extent in the late autumn, and all but absent in the spring (*cf.* Kylin). There is also some evidence to show that it exists in the weed in the form of an ethereal sulphate complex. The presence of carbohydrate ethereal sulphates in marine algae has already been shown by Hoagland and Lieb (11) and Bird and Haas (12), and more recently Lunde and Heen (13) have shown that the drops of liquid exuding from *L. digitata* contain a mucilaginous material which consists largely of fucose combined in an ethereal sulphate complex. When laminariae fronds are exhaustively extracted with boiling water until the extract is free from sulphate, and mucilaginous materials have been removed, the fronds when allowed to stand for some days with dilute acid yield some laminarin. No sulphate ions appear in the acid extract until the latter becomes milky. Free sulphuric acid, however, appears at the same time as the laminarin, and the quantity increases as the milkiness of the extract develops. It is also of significance in this connection that in the preparation of the laminarin the white deposit makes its appearance sooner when hydrochloric acid is employed than when other acids are used. Hydrochloric acid behaving generally as the strongest acid, this would be expected if a sulphate complex of laminarin is being slowly decomposed in the cold with the breaking off of the sulphuric acid residue. The probability is, therefore, that some at least of the laminarin is present in the weed as an ethereal sulphate.

Experimental.

Preparation of Laminarin.—L. digitata fronds, collected on the 9th day of September at Spiddal, Co. Galway, were dried in the steam-oven until brittle, and then passed through a laboratory cutter. 260 g. of the finely divided product were covered with dilute hydrochloric acid (5 c.c. concentrated hydrochloric acid per 100 c.c. distilled water) and allowed to stand. After four days the mixture was well shaken and the milky liquid decanted and allowed to stand for two days. Most of the laminarin had deposited in this time. A further quantity was separated on the centrifuge, the liquid separated being still milky, however. A second extraction was performed in a similar manner. The combined laminarin fractions were dissolved in hot distilled water, and the filtered solution allowed to stand for a few days when the solid had deposited in white flakes. The liquid was decanted, and the laminarin dissolved again in hot water and allowed to deposit spontaneously. After two further depositions, the laminarin was shaken with absolute alcohol and collected on a glass filter and washed with alcohol and ether. After drying on the filter and in a vacuum desiccator over sulphuric acid, it was obtained as a fine snow-white powder containing less than 0·2 per cent. ash. It weighed 18·5 g.

Attempted Separation of Laminarin into Different Fractions.—100 c.c. of about a 6 per cent. aqueous solution of pure laminarin were allowed to stand for

five days and the liquid decanted each time from the laminarin deposited on successive days. The different deposits were dissolved separately in warm water, and the rotatory power and concentrations of the solutions measured. A typical estimation is given here:—

A solution containing 2·186 g. per 100 c.c. (Ash: 0·46 %) gave a rotation of −0·42° in a 2 dm. tube.

$$\left[\alpha\right]_D^{10^\circ} = -\frac{42}{4{\cdot}352} = -9{\cdot}85^\circ.$$

Variation of $\left[\alpha\right]_D^{10^\circ}$ *with Concentration.* — The laminarin used in these estimations was dried at 105° C. under a pressure of 1·5 mm. for four hours. A weighed quantity was then dissolved in hot water to a perfectly clear solution. Having measured the rotation of the cooled solution, it was diluted by successive additions of 5 c.c. distilled water to 10 c.c. of the solution. After each addition of distilled water the rotation was measured. The figures given in Table 2 show that dilution of the solution 10 times results in the doubling of the specific rotatory power.

Estimation of the Molecular Weight. — The estimation of the molecular weight was carried out by measuring the iodine consumed by 25 c.c. of a solution of laminarin of known concentration when allowed to stand for four hours with excess $N/10$ iodine in the presence of sodium hydroxide. The following is a typical example: 25 c.c. of laminarin solution containing 0·544 g. were allowed to stand for four hours with 5 c.c. 1·0419 $N/10$ iodine and 7·5 c.c. $N/10$ sodium hydroxide. The mixture was then acidulated with dilute sulphuric acid, and the excess iodine titrated with 0·9737 $N/10$ sodium thiosulphate (3·50 c.c.). A blank required 5·38 c.c. of the thiosulphate. Thus 20,000 c.c. $N/10$ iodine would oxidize 5913 g. of laminarin. A series of determinations made with solutions of different concentrations showed no uniform change of the molecular weight with the concentration, the results generally falling between 5000 and 6000 (Table 3). As the oxidation was carried out in an alkaline solution it was presumed that no change in the molecular size took place during the experiment. The laminarin recovered from the oxidation had not lost its property of spontaneous precipitation.

Hydrolysis with Sulphuric Acid.—50 c.c. of an aqueous solution of laminarin containing 0·7203 g. were heated on a boiling water-bath for four hours with 2 c.c. concentrated sulphuric acid. The liquid was cooled and again made up to 50 c.c. The rotation of this liquid was +1·495°. This corresponded to 0·7115 g. of glucose. Theoretical yield of glucose is 0·8003 g.

Hydrolysis with Hydrochloric Acid.—50 c.c. of the same laminarin solution were heated on a boiling water-bath for 2½ hours with 6 c.c. of concentrated hydrochloric acid. The cooled liquid made up to 50 c.c. gave a rotation of +1·653°. This corresponds to 0·7877 g. of glucose, *i.e.*, 98·5 per cent. conversion to glucose.

The Mode of Occurrence of Laminarin.—A quantity of ground fronds was covered with distilled water and boiled for five minutes. The extract was poured off and the extraction repeated, four times in all. The first two extracts contained sulphate ions; the third and fourth extracts did not contain any. The residual fronds were now washed with distilled water and covered with dilute hydrochloric acid (6 c.c. concentrated acid to 100 c.c. distilled water). After three days the acid was slightly milky and when filtered clear gave a turbidity with barium nitrate solution and dilute nitric acid. On further standing a deposition of laminarin occurred, and the barium sulphate precipitate became considerable in quantity. In a second experiment the fronds were boiled six times with water before allowing them to stand with the dilute acid. Deposition of laminarin in this case was evident after twenty days, and not until then could the presence of sulphate ions be shown in the extract.

Derivatives of Laminarin.—Laminarin can be acetylated and methylated simply by the usual methods employed in dealing with polysaccharides. These will be described in a future communication. It is of interest that laminarin, acetylated by heating with acetic anhydride and sodium acetate and subsequently recovered by saponification with alcoholic potash, does not appear to have lost the power of spontaneous precipitation.

SUMMARY.

1. A survey has been made of the literature dealing with laminarin, and points that were in doubt as to its composition have been examined.

2. It has been shown that laminarin consists exclusively of glucose, when hydrolysed by dilute hydrochloric acid. 100 per cent. conversion to glucose is not effected by heating with dilute sulphuric acid.

3. Kylin's assertion that laminarin is a mixture of closely related polysaccharides of different degrees of polymerization has not been confirmed. It has been shown that laminarin is an unstable colloid whose rotatory power increases uniformly with dilution. This appears to be due to a decrease in the size of the colloidal molecule.

4. Measurements of the consumption of iodine in an alkaline medium by solutions of laminarin of different concentrations show no uniform change with increasing concentrations. This would indicate that no change is taking place in the size of the chemical molecule, and that the variations in rotatory power are due to physical aggregation and deaggregation.

5. A simple method for the isolation of pure laminarin from *L. digitata* fronds is described.

6. Evidence is given which indicates the possibility that laminarin occurs in the weed as an ethereal sulphate complex.

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No. 57.

CONIDIA FROM INFECTED BUD-SCALES AND ADJACENT WOOD AS A MAIN SOURCE OF PRIMARY INFECTION WITH THE APPLE SCAB FUNGUS *VENTURIA INAEQUALIS* (COOKE) WINT.

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(Plate 19.)

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Introduction.

In the apple scab fungus (*Venturia inaequalis*) the two important sources of primary infection which have usually been considered are:—

(1) Conidia from pustules which develop during winter and early spring on the one-year-old wood,

(2) Ascospores liberated from perithecia formed on dead infected leaves of the previous season.

Both sources occur apparently wherever the apple is cultivated and scab a major problem, but the importance attached to each varies considerably in different countries. This is no doubt due to the prevailing climatic conditions and also to differences in the varieties grown. In Europe scabbed wood has long been known, and on certain varieties more or less of it occurs every year. In seasons favourable to the disease it is prevalent in Ireland, and in such years the current season's growth of any variety may be attacked, develop pustules, and consequently form a serious source of infection in the following spring. Scabbed wood is not considered of much consequence in America, where the principal source of infection is believed to be ascospores from the dead overwintered leaves. In Australia and New Zealand about equal importance appears to be attached to each of these sources.

In 1931 Salmon and Ware (16) drew attention to the fact that in some cases neither of the above sources of infection was present, and they recorded for the first time scab pustules on scales of blossom buds of Worcester Pearmain, and found them responsible for the early outbreak of the disease on the young leaves surrounding the blossoms.

During the investigation of apple scab at Glasnevin cases of severe early spring infection on the foliage of spurs have been seen each season, where, as in the case of Salmon and Ware, neither scabbed shoots nor apparently dead leaves were involved. Indeed the problem of the early occurrence of apple scab each year on isolated Bramley's Seedling has been an unsolved puzzle. Scabbed wood does not usually occur on this variety, though it was found for the first time on unsprayed trees of Bramley's Seedling in 1936. It is generally considered that ascospores from overwintered leaves are the source of infection in this and other similar cases, but the orchard at Glasnevin is cultivated and no perithecia were found. This is not to say that perithecia do not sometimes mature in apple leaves in this country, for ascospores were found to be produced at the rate of over 11,500 per sq. cm. of leaf surface in 1926, beginning as early as February 19th, but their formation is generally erratic, and sometimes none are produced.

That in many instances infection arises in the region of the buds themselves, and that this source, so long overlooked, occurs in many varieties, was shown by a systematic examination of dormant buds during the early months of 1937 and by the subsequent development of the disease in the spring of the same year.

Infection in the Bud Scales.

Apple scab was exceptionally severe in the College orchard in 1936. Trees of Bismarck and of Bramley's Seedling, which had been left unsprayed for scab for the second season, developed 99 and 87 per cent. respectively of scabbed fruit, and badly attacked foliage as well. In January, 1937, a microscopical examination of the dormant buds of the unsprayed Bismarck showed 53 per cent. of them to contain viable scab mycelium in the bud-scales and in the woody tissue contiguous to the scales. This was not surprising in the case of Bismarck, as it is notoriously liable to scab, and some such hidden source of infection has been suspected, since neither scabbed wood nor fallen leaves provided sufficient explanation of the recurring early and severe attacks.

The fruit buds of Bramley's Seedling, however, proved to be almost as bad, for an average of 49 per cent. of them on the unsprayed trees were infected in a similar manner. Further examination of the buds of other varieties, which had been sprayed three times with lime-sulphur in 1936, also showed a considerable number of them to be similarly attacked.

It should be noted that all these trees had been sprayed in the winter of 1936–1937 with a recognised tar-oil-wash. This was evidently of no effect in controlling the development of the apple scab fungus during the dormant period.

In January or February no external evidence of the presence of infected buds could be seen, and the spurs with the attached buds were collected at random from the centres of the trees. If the bud is taken at its apex and bent over it separates easily at its base, leaving the outer and lowermost scales attached to the stem. Contiguous to the scales there is a tissue which is mainly collenchymatous, and whilst infection is frequent in the bud-scales themselves it is also very prevalent in this soft tissue at their base. In the early months of 1937 sections, taken through the outermost bud-scales and through the soft tissue contiguous to them, revealed mycelial masses of the scab fungus developing in both places in the following varieties:—

*Allington Pippin.
Annie Elizabeth.
*Bismarck.
*Belle de Pointoise.
*Bramley's Seedling.
Coronation.
*Cox's Orange Pippin.
*Domino.
Ecklinville Seedling.
*Emperor Alexander.
*Felthom Beauty.
Irish Peach.
James Grieve.
Kentish Fillbasket.
King Edward VII.
*Lord Grosvenor.
Newton Wonder.
Royal Jubilee.
*Thomas Rivers.
Tower of Glamis.
*Warner's King.
*White Astrachan.

The following additional varieties were found with scab infection in their bud-scales in November, 1937:—

Galloway Pippin.
Gibby.
Golden Harvey.
King of Tompkins County.
Sandringham.
Wellington.

In the first list the number of infected buds varied from 3 per cent. to over 50 per cent., and in those marked with an asterisk some of the scab cushions were producing conidia before 9th March, 1937. Incidentally twelve of these varieties showed scabbed one-year-old wood, but no infected wood could be found on the other ten except what occurred at the base of the bud-scales. The percentage of infected buds did not appear to bear any relation to the amount of scabbed wood present, and this was particularly so in the case of Bramley's Seedling.

Buds, to the average number of 50, of several other varieties were also examined but were found negative. This is no proof that they never harbour the fungus, for apple scab had not been prevalent on them in 1936, and the negative varieties included Worcester Pearmain, on which the original discovery had been made by Salmon and Ware (16).

In cross-sections of infected buds made in February the mycelium of the scab fungus is easily recognised under the microscope as small pads or cushions of hyaline, closely packed, hyphal cells, lying usually just below the outer tissue of the bud-scales, but occasionally deeper in the host tissues, especially at the base of the scales. There is frequently more than one point of infection (figs. 1 and 4), and cases were seen where the infection was spreading from one bud-scale to another or from the base of the scale into the stem.

These lesions are variable in size, but they stand out distinctly from the host cells, as in addition to being hyaline the fungal cells are much smaller. In their early stages there is only a little group of hyphal cells present, but in most varieties by the early part of February the mycelial masses are from ¼ to 1 millimetre long parallel to the surface of the host, and from 20 to 200μ or more thick, forming a well-marked stroma. As development proceeds, the outer cortical cells of the host become torn away from the tissues beneath, raised up (fig. 2), and eventually ruptured. At the same time the stroma becomes cushion shaped, the hyphae being somewhat radially arranged, and, finally, the characteristic conidia of the fungus are formed on its exposed surface in a dense mass (fig. 3). In surface view the outlines of the pustules vary from circular to elliptical. The size of the stroma depends more or less on the variety, but in the majority of cases the longest diameter was slightly less than one millimetre. The size in the variety Lord Grosvenor was exceptional, being up to 5 mm. in diameter by 1·5 mm. thick, and sometimes encircling one-third of the circumference of the top of the spur.

Infected bud-scales were recorded by Salmon and Ware on blossom buds, but they were also found in the present work on foliage buds, though the former were by far the most numerous. Sporulation occurred naturally as early as the second week in February, but the majority of the fungal stromata remained covered and sterile during this month. Sterile but well developed stromata were easily induced to form conidia by cutting sections through them and leaving them in tap water for 48 hours at room temperature.

The majority of infected bud-scales were producing conidia from the middle of April onwards until they were abstricted, but these centres of infection could seldom be detected without microscopical examination. Thus, on the 14th April, just before the buds were in the mouse-eared stage, 25 blossom buds from one of the severely infected Bramley's Seedling trees were examined, but in one case only was the disease detected, even with a lens. Microscopic examination showed that 12 of these buds had infected scales, and 9 of them were producing conidia either on the scales or just below them at this time. Active centres of infection were, therefore, present on 36 per cent. of the blossom buds in the centre of this tree.

Usually the scab pustules develop on the outside of the bud-scale, but occasionally they form on the inner surface, and in one instance a pustule was found on the pedicel of the blossom truss within the bud.

First Appearance of Scab on the Foliage and its Subsequent Development in 1937.

The first infected foliage of the year was found on May 5th on a tree of Bismarck which had shown a severe late attack of scab during August, 1936, and many infected bud-scales when examined microscopically during the following winter. Some of the pustules were fructifying freely on the scales in April, and the first diseased foliage and flowers occurred under circumstances which indicated infection before or during emergence. The first infected spur noticed had four out of the six leaves surrounding the blossom-truss diseased, one of which was producing conidia on the upper, and the others on their lower surface. Two of the leaves were distorted and covered with scab for over one-fourth of their surface, and two of the diseased leaves were not completely unfolded. Scab spots, more or less hidden by the thick woolly growth of hairs, were also found on the unreflexed sepals. Several spurs with similarly infected foliage and blossoms were found a few days later on the same tree.

Other spurs with similar little groups of diseased leaves were found for the first time on 11th May on Allington Pippin, Annie Elizabeth, and on three unsprayed Bramley's Seedling trees, at a time when they were all in the pink bud stage. On Bramley's Seedling the disease was just showing as a slight darkening of sometimes the upper and sometimes the lower surface of the leaf. It was so indefinite that microscopical examination was necessary, this proving that the fungus was already sporulating. Allington Pippin showed the most disease, and the infection occurred chiefly on the backs of the leaves. The infection was confined in every case to the oldest leaves of the whorl, a number of which were producing conidia at the time, and usually 50 per cent. at least of the whorl of leaves was affected. The fact that neighbouring foliage was healthy at the same time supported the view that infection arose principally in the region of the buds, and examination showed such buds to possess infected bud-scales.

The percentage of leaf whorls infected at that time was, however, very small in comparison with the number of buds with infected scales which previous examination had shown to be present. It is believed that all these cases were probably due to infection which took place before the leaves emerged. Observations showed that most of the scab which subsequently developed on the foliage began during and after the emergence of the leaves, copious infection taking place throughout the flowering period and after petal fall.

By the end of May many of the early-attacked leaves were distorted by the disease, which was so severe on unsprayed Allington Pippin trees that 25 per cent. of their leaves was scabbed, this figure rising to over 80 per cent. by the end of June. On control Bismarck trees 10 per cent. of the leaves was diseased at the end of May, and 75 per cent. by the end of June. The development of the disease on control Annie Elizabeth was not quite so rapid, but

here again 75 per cent. at least of the foliage was showing scab by the middle of July. In all cases the fruit was badly spotted by the disease at the same time.

In the case of Bramley's Seedling, the number of infected buds was found to vary in relation to previous spray treatment, and further work was therefore concentrated on it in 1937. There were in all 26 trees of this variety over 30 years old, growing in two lines, the latter 8 yards apart, which had all received the same treatment, consisting of a tar-oil winter wash and several applications of 3 : 9 : 40 Bordeaux mixture in spring up to 1934, inclusive. During 1935 and 1936 these trees were divided into four plots, which received various fungicidal treatments, and these were separated from each other by three plots consisting of two unsprayed trees, one in each line. The control trees suffered from a severe scab attack in 1936 and had only 12·5 per cent. of clean fruit, compared with 76·8 per cent. on the sprayed trees.[1]

This was correlated in the ensuing winter with a high proportion of buds with infected bud-scales on each of these six untreated trees, the percentage varying from 42 to 56. One neighbouring sprayed tree from each of the four plots when examined at the same time showed 0 to 2 per cent. of similarly infected buds.

In consequence of this it was decided to spray one of each pair of controls in 1937, and to leave one of the previously sprayed trees untreated instead. There were, therefore, available for observation in that year three groups of three uniform Bramley's Seedling trees standing close together in the form of three triangles, together with one additional tree from the fourth sprayed plot, on which the percentage of buds with infected bud-scales was known, as follows :

No. of trees.	1936.	Per cent. infected buds.	1937.
4	Sprayed	0–2	Unsprayed
3	Unsprayed	42–46	Unsprayed
3	Unsprayed	48–56	Sprayed

The results are shown in Table 1. The trees are arranged there as they occurred adjacent to each other in the rows, and they are best considered in groups of three.

In the first place it is clear that there is a high correlation between the amount of scab in 1936, as shown by the percentage of diseased fruit (Col. 5) and the percentage of infected buds in the following winter (Col. 7).

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[1] The full results of spraying these trees in 1935 and 1936 will appear shortly in the Journal of the Department of Agriculture.

There is also a direct relation between a high percentage of infected buds and the early appearance of scab, on the one hand, and between absence or virtual absence of infected buds and the late appearance of scab on the other (Cols. 10 and 11), in all cases in which the development of the disease was not hindered by spraying, that is, in the three pairs of trees which were not sprayed in 1937 (Nos. 1 and 3, 4 and 5, 7 and 8), and also on the extra tree No. 10.

Early appearance of the disease was in all cases followed by rapid spread on the foliage of the same tree in May and June, as compared with late appearance (Cols. 11 and 12), and this is, therefore, a further result of the presence of many infected buds.

It is evident that a close direct relation exists between the amount of infected foliage and scabbed fruit, even though yields in general were low compared with that of the previous year.

The absence of infected buds was of such paramount importance that its effect in seasons like 1937 in checking scab on the foliage, not only initially (Col. 11) but throughout the season (Cols. 12 and 13), and even on the crop as late as October (Col. 15), approached in most cases that of three or four sprays.

Each tree provided its own source of infection in 1937. It is clear from the foregoing conclusions, and especially from a consideration of the seven trees which were not sprayed in 1937, that the chief source of primary infection developed on the tree itself and not outside it.

Infection from ascospores is ruled out in the case of this experiment for the following reasons:—

(*a*) No dead leaves were present around the trees;

(*b*) Had ascospores been present to any extent a serious amount of disease would have been expected also on unsprayed trees in the absence of infected buds, but such did not develop in any case.

Observations on King Edward VII and Gascoyne's Scarlet Seedling in 1937 provided further confirmation of these conclusions. As a rule both varieties take scab severely at Glasnevin when unsprayed. There were two trees of King Edward VII, one of which was practically free from scab during 1936 and showed no bud infection when examined during the winter, and the other on which the foliage and subsequently the buds were severely infected. Both were left without treatment in 1937, with the result that the tree with the healthy buds produced 132 lb. of fruit, of which 3·6 per cent. was scabbed, and the other 35 lb. of fruit, of which 37 per cent. was scabbed. A single tree of Gascoyne's Scarlet Seedling, which was free from scab in 1936 and showed no infected buds subsequently, was not sprayed in 1937 and produced 117 lb. of fruit which showed only 17 per cent. of scab.

The attack of scab was severe in the spring of 1937, a fact which is in harmony with the foregoing results, for the disease had been prevalent in 1936 and many infected buds were found during the winter, as stated. Climatic

TABLE

Showing Relation between Spray Treatment, Infected Buds, Appearance, and Spread of

Tree No.	Spray treatment, 1936.		Total Yield, 1936.	Scabbed Fruit, 1936. Per cent.		Buds with infected scales, Feb., 1937.	Spray treatment, 1937.		Date on which scab was first found.
	Pre-blossom.	Post-blossom		Total.	Severe.		Pre-blossom.	Post-blossom.	
			lbs.			per cent.			
1	1 Lime-sulphur 1/30.	2 Lime-sulphur 1/80.	567	23	1·6	0	None.	None.	24 May,
2	None.	None.	377	95	76	48	1 Lime-sulphur 1/30.	2 Lime-sulphur 1/80	31 May
3	None.	None.	659	90	65	45	None.	None.	14 May,
4	2 Bordeaux mixture. 3 : 10 : 40	2 Bordeaux mixture. 3 : 10 : 40.	321	0·9	0	0	None.	None.	1 June.
5	None.	None.	607	78	57	46	None.	None.	11 May.
6	None.	None.	117	80	55	48	2 Bordeaux mixture. 3 : 9 : 40,	2 Lime-sulphur 1/80	No record.
7	2 Lime-sulphur 1/30	1 Lime-sulphur 1/80.	644	55	17	2	None.	None.	31 May.
8	None.	None.	200	90	62	42	None.	None.	11 May.
9	None.	None.	263	93	66	56	2 Lime-sulphur. 1/30.	2 Lime-sulphur 1/80	22 June.
10	1 Hydrated lime Bordeaux mixture. 8 : 12 : 100	2 Hydrated lime Bordeaux mixture. 8 : 12 : 100	417	2	0	0	None.	None.	1 June. One leaf only.

The dates 31 May and 22 June are the

1.

Apple Scab, and Control due to Spraying on Bramley's Seedling, 1936–1937.

Observations on infection of foliage, spread, and development of the disease in 1937.			Total Yield, 1937.	Clean Fruit, 1937.	Scabbed Fruit. 1937. Per cent.	
31 May.	22 June.	22 July.			Total.	Severe.
			lbs.	per cent.		
Scab present on a few scattered leaves.	About 1 per cent. of leaves infected.	5 per cent. of all leaves attacked.	218	89·4	10·6	3·7
Three infected leaves found.	Scabbed leaves less than 0·5 per cent.	Little spread of the disease. Infected leaves less than 1 per cent.	219	90·1	9·9	2·8
Scab present on 4 per cent. of all leaves.	50 per cent. of leaves attacked, and 90 per cent. in heart of tree, considerable defoliation.	Over 80 per cent. of the leaves scabbed.	174	56·5	43·5	19·8
—	Scab outbreak on one side of tree, that next No. 5. Total leaves affected less than 0·5 per cent.	Less than 1 per cent. of all leaves attacked.	113	95·4	4·6	2·2
Scab fructifying on 5 per cent. of leaves.	70 per cent. of leaves attacked, and in centre of tree over 95 per cent. scabbed. Some defoliation.	90 per cent. of leaves scabbed.	169	44·2	55·8	27·2
No record.	An occasional infected leaf.	Less than 0·5 per cent. of leaves attacked.	40	92·0	8·0	4·3
An occasional scabbed leaf, less than 0.5 per cent.	6 per cent. of leaves attacked.	8–10 per cent. of leaves with scab fungus.	161	77·6	22·4	9·6
Scab fructifying on 4 per cent. of leaves.	Over 50 per cent. of leaves scabbed, with premature defoliation.	Over 75 per cent. of leaves scabbed.	127	39·6	60·4	33·1
—	A few scabbed leaves only seen.	Less than 0·5 per cent. of leaves attacked.	208	98·2	1·8	0·2
—	Less than 0·5 per cent. of leaves attacked.	Infected leaves still below 1 per cent.	158	96·5	3·5	1·3

times of post-blossom spraying, 1937.

conditions also favoured the disease during May and June, 1937, the weather being cold and sunless, though dry. (Table 2.)

TABLE 2.

Number of days on which precipitation occurred, and rainfall for each month, April to September, inclusive, 1936 *and* 1937.

Year.	APRIL.		MAY.		JUNE.		JULY.		AUGUST.		SEPTEMBER.	
	No. of days.	Rain-fall inches.	No. of days.	Rain-fall inches.	No. of days.	Rain-fall inches.	No. of days.	Rain-fall inches.	No. of days.	Rain-fall inches.	No. of days.	Rain-fall inches.
1936	17	1·91	12	1·33	17	3·08	27	5·24	13	1·14	16	2·83
1937	16	1·80	14	1·34	14	1·24	17	1·96	11	2·69	19	1·45

Where infected buds were numerous and no spray was used, scab appeared early and spread rapidly, and the effect on Bramley's Seedling in particular was so severe as to cause considerable defoliation in the second half of June. Local conditions did not favour the further spread of the disease from mid-July to mid-September, but after that it became active again and conidia were being produced during the remainder of the season.

The clear-cut results obtained from this experiment were largely due to the weather of 1937, for if July had been as wet as it was in 1936 much more disease would have probably developed on those trees (Nos. 1, 4, 7, and 10) on which the number of initially infected buds was the limiting factor, an event which might have somewhat obscured the result.

A comparison of the same four trees indicates a difference in fungicidal power between Bordeaux mixture and lime-sulphur, and shows that this may make itself felt over two seasons. The Bordeaux mixture, although unfortunately it injured the foliage and russeted the fruit, practically eliminated scab from both foliage and fruit in 1936 (trees 4 and 10, Table 1), and a further effect was that no infected buds could be found on either tree during the winter. The result was that the total amount of scab in 1937 amounted only to 4·6 and 3·5 per cent. respectively, even in the absence of spraying. The control of scab given by lime-sulphur on trees 1 and 7 in 1936 was much poorer both on the fruit (Table 1, Col. 5), and also on the foliage. Tree No. 7 showed 2 per cent. of infected buds during the winter, and a moderate attack subsequently developed on the leaves, and ultimately involved 22 per cent. of the crop. The other tree (No. 1) probably had a few infected buds also, though none were found, for it had scab on the leaves in the previous autumn, and it developed somewhat more of the disease on both foliage and fruit in 1937 than the corresponding trees sprayed in 1936 with Bordeaux mixture.

Nevertheless, in a year like 1937 lime-sulphur as well as Bordeaux mixture, when applied three or four times, effectively controlled the disease, notwithstanding the presence of many infected buds.

Period during which the Bud-scales continue Infective and Infection of the New Scales.

Scab-infected bud-scales provide a source of infection extending from February until they are completely thrown off, with the maximum formation of conidia occurring towards the end of April and throughout May and June.

Scab pustules on the shoots immediately below the bud-scales were fructifying as late as 15th July on the varieties Allington Pippin and Bismarck. On this date the pustules, although almost severed from the host tissue beneath by the formation of a new cork layer, were still brown to black in colour, and carried abundance of conidia, but on Annie Elizabeth and Bramley's Seedling they were completely severed from their host, dried up, and sterile.

On the new buds the first infected scales were found on the 3rd July on Allington Pippin, and a few days later on Bramley's Seedling. On these immature newly infected scales the fungus did not form the well-developed stromata, which were so characteristic a feature underneath the outer tissues in the mature scales. Instead, the conidiophores grew out without the development of stroma, just as occurs on ordinary foliage. This attack on the young bud-scales which were still green could be easily recognised with the naked eye owing to the black lesions, and the fungus was found fructifying on current season's scales up to 29th September. The first well-developed stroma beneath the outer tissue of a bud-scale was found on the 3rd September—this, however, appeared to be exceptional, as one only was found. Early stages in the development of the stroma were found in bud-scales of Bismarck on 22nd October, the hyphae forming a few tangential strands on the outer edge of the cortex. Similar stages of infection were found in bud-scales, previous to leaf-fall, of several other varieties which had severe scab attacks on their foliage in 1937.

Observation of Scab Attacks on the Young Wood.

Owing to the severity of apple scab in the wet season of 1936 many varieties that year developed scab pustules on the current season's growth, on which their development had not hitherto been reported. Amongst these was Bramley's Seedling, several trees of which showed well-developed scab pustules which were producing conidia by the end of November. The majority of these infected shoots were removed as far as practicable during winter pruning, but on one tree a number of them were left for observation purposes. Conidia were being produced on the pustules of these shoots from November, 1936, until the end of the first week in July, 1937, but were most abundant from March until the

middle of June, more spores being produced than the previous appearance of the pustules would have indicated. Cracking and scaling of the bark of the attacked shoots occur in Bramley's Seedling just as in the other varieties.

On the 20th August, 1937, an examination of an unsprayed orchard in the north of the country showed scab-attacks on the current season's wood of 19 out of 30 Bramley's Seedling trees. The amount of scabbed wood present on each tree was not great, but the pustules were already well formed. The same feature was again found on two unsprayed Bramley's Seedling at Glasnevin on 2nd September, 1937, on which date a few of the pustules were on the point of bursting. Scab attacks on the young wood of Bramley's Seedling, therefore, appear to be of more general occurrence than previous observations indicated.

On the 14th July, 1937, an outbreak of scab occurred on the foliage of a group of young shoots and on the nearby fruit, on a bush tree of Annie Elizabeth. This tree had received two pre-blossom and two post-blossom sprays of lime-sulphur, the last being on 21st June, on which date no scab was apparent on the foliage. As the tree was partially isolated and was separated by two others of the same variety from an unsprayed tree, it was surmised that the attack was coming from the tree itself. On examination it was found that scabbed one-year-old wood occurred right in the centre of the outbreak. At this time many of the scab pustules were completely severed from the host by the formation of new cork tissue, but on closer examination numerous small swellings were found on the bark, which opened by a mere slit and were giving rise to conidia even though the shoots were covered with spray material.

A week later a similar outbreak of scab was found in the middle of a young tree of Laxton's Superb, and here again scabbed one-year-old wood occupied the centre of infection, and very small swellings on the back were liberating conidia.

An examination of other trees showed infected one-year-old shoots of Bismarck, Cox's Orange Pippin, Golden Harvey, Kentish Fillbasket, and Wellington to be producing conidia as late as 29th July, 1937. At a casual glance the scab pustules appeared as if they were completely thrown off, and this was true of the larger ones, but, as before, further investigation showed very small swellings elsewhere on the bark. It was found that these minute lesions could be easily detected if the shoots were taken and immersed in water for a few minutes, when the black velvety mass of conidia were then easily seen in the slit-like opening in the little swelling.

Scab attacks on the current season's growth of Golden Harvey have a similar effect to that produced on Cox's Orange Pippin, namely, premature defoliation of either the top leaves or those on the middle of the shoot. Not only were conidia being produced on the one-year-old wood of Golden Harvey, but small pustules were already formed and were fructifying on the current season's growth, the latter at this time being two feet long. There was thus a distinct overlapping of conidial production by scab pustules on the one-year-old wood and on the current season's growth.

Discussion.

In the case of the apple scab fungus, both in Europe and America, the principal source of primary infection in spring has been a controversial subject amongst research workers. Marsh (12) has definitely shown the part played by scabbed one-year-old wood, and he points out that some winters are extremely favourable for the complete destruction of fallen leaves, thus practically eliminating ascospore development; and he states, "that the hypotheses, based on the work of Keitt and others in U.S.A., concerning the importance of infection from the over-wintered leaves, ought not to be accepted for this country without further examination." Support for Marsh's opinions is given by the observations of Dillon Weston and Petherbridge (2), who came to the conclusion that ascospores played no part in the initial infection of apple or pear scab in the orchards under their observation in 1932. A similar conclusion was reached by Goosens (9) in Holland, where it was shown that the part taken by ascospores was evidently subordinate, and that the primary source of apple and pear scab was mainly over-wintered conidia.

On the other hand, in America, though scabbed wood has been recorded by numerous observers, its rôle is considered negligible, except on a few varieties and under certain conditions, and ascospores are stated to be the source of primary infection. The prevention of ascospore development by ploughing-in of the fallen leaves can be readily understood, and it has been recommended. Wallace (18) believed in the efficacy of ploughing-in the fallen leaves, and records observations in its support, but Glover (7) reports on the futility of this procedure, and writes, "orchards have been repeatedly plowed in the autumn, so that no dead leaves were in sight the following spring. In no case was it evident that the plowing lessened the amount of scab on the trees the following year." Recent reports by Folsom (3) and by Glover (7) indicate that more attention is now being paid to the rôle of scabbed wood in America. Evidence in support of the importance of ascospores in Europe is supplied by the observations of Salmon and Ware (15, 17) and Beaumont and Hodson (1) from England. Muskett and Turner (14) believe the ascospores to be the most important factor in the over-wintering of the disease on Bramley's Seedling in Ireland. Marsh and his co-workers (13) are prepared to accept the view that ascospores must be the source of primary infection in those varieties not susceptible to shoot attack. Data obtained by Holz (11) in Germany, and by Wiesmann (19) in Switzerland are considered to substantiate the observations of American and European workers on the primary importance of ascospores.

Many more references could be quoted on both sides of the question regarding the part played by scabbed wood and by ascospores respectively. Those given may, however, be taken as representing our present state of knowledge on this subject, and it is evident that further investigation is required.

Of the numerous observers who have reported on severe early attacks of

apple scab on the blossoms and especially on the leaves surrounding the flower trusses, Salmon and Ware (16) have been the only ones who ultimately connected such an attack with its true source. A short note by Gloyer (8) on the part taken by over-wintering conidia in initial infection, is very suggestive in relation to the facts presented in the present paper.

Parasitized bud-scales and contiguous tissue are a much more insidious source of infection than scabbed one-year-old wood, for as a rule they cannot be recognised macroscopically except in rare cases. This probably accounts for their being largely overlooked up to the present, and to the scant attention given to the original observation of Salmon and Ware (16).

Infected bud-scales occur on trees along with scabbed wood, but they can be quite numerous on trees in absence of the latter. Infected bud-scales and adjacent tissue were responsible for the early appearance and subsequent serious development of the disease on trees of several varieties at Glasnevin in 1937, such as Bramley's Seedling, in the absence of both scabbed wood and dead leaves.

Scabbed shoots were not a factor of any consequence in initiating the disease in 1937 on the foliage of the Bramley's Seedling trees referred to in Table I. On tree No. 3 eight diseased shoots were present, and they were left for observation purposes, this being the first occasion that scabbed wood was seen on this variety. Their presence did not appear to influence the development of the disease to any extent, for the first infected foliage at the base of these shoots was not found until 24th May, that is, ten days after it had been seen elsewhere on the same tree.

On varieties susceptible to shoot infection, the importance of scabbed wood is well recognised. Folsom and Ayers (4) found still unbroken twig pustules on June 13th, and they state:—"The twig pustules can, and usually do, cause infection of the leaves later in the season than ascospore infection of leaves occurs." Conidia from this source may be responsible for late outbreaks of scab on trees which were well sprayed in the earlier part of the season, as is described here for Annie Elizabeth and Laxton's Superb. Infected shoots of many varieties were producing conidia up to the end of July at Glasnevin in 1937. This confirms the observations of Dillon Weston and Petherbridge (2), for they found pustules on one-year-old growths of the spur discharging spores from May until August, when observations ceased.

Very little spread of the disease occurs from tree to tree, even where branches practically touch, as was the case with trees Nos. 4 and 5, Table 1. Considering the proximity of these and the severity of apple scab on No. 5, the small amount of spread was remarkable. This is in agreement with other investigators (2, 6, 7) who have found that scab once established on a tree is more or less limited to it, the conidia produced serving mainly for dissemination within the tree. Frey and Keitt (5) have shown that the conidia of *Venturia inaequalis* are very resistant to detachment from their conidiophores when dry, but quickly become detached in the presence of water, and are, therefore,

disseminated chiefly in meteoric water acting under the influence of wind and gravitation.

When the disease was kept well under control there was a very definite cumulative effect from spraying in the season following the applications, but this persisted for one year only at Glasnevin. Trees of Allington Pippin and Bramley's Seedling which were practically free from scab at the end of 1934 remained comparatively clean in 1935, but were all exceptionally bad with the disease in 1936. No spraying for scab was done on these trees in either of the latter years. Similarly, sprayed trees which were clean in 1936 remained remarkably clean in 1937 without any scab treatment (Nos. 2, 4, 10, and to a less extent No. 7, Table 1). This supports Marsh's (12) statement that:—"Where trees have been practically freed from such scab in one year it is possible to take considerable liberties with the spraying programme in the following year and still obtain satisfactory results." Grubb (10) previously reported that the effect of fungicides was not confined to the season of application, but in some cases probably extended into a second and a third year. Now it is fairly obvious that the extension of any cumulative effect beyond the first year will depend on two main factors, firstly, the relative freedom of the trees from scab in the year when spraying ceases, and, secondly, the weather conditions in the following years. If conditions are not favourable to scab during the first year, in the absence of any spraying the trees may remain healthy, and the beneficial effects extend to the second or third season.

Under the conditions of the experiment the cumulative effect of spraying is a further argument in favour of the minor importance to be attached to infection arising from ascospores. In practically all experiments on apple scab control a number of trees are always left unsprayed, and the diseased leaves from these trees must be scattered about amongst all the others during the winter. Such being the case, then, it is very difficult to account for the disease being worse on trees which have been unsprayed for several seasons, than on those left unsprayed for the first time, unless infection is coming from the bud-scales and other sources on the tree itself.

Further investigation is required on the following:—

(*a*) The part played by late dormant sprays, which have been recommended elsewhere;

(*b*) The part played by the first pre-blossom spray in scab control, *i.e.*, that at the mouse-eared stage;

(*c*) The importance of ascospores.

On trees where scab has been severe two pre-blossom sprays appear necessary, the first being an essential one, whereas where the disease has been kept under control the first pre-blossom spray can apparently be dispensed with.

For the successful control of apple scab, more attention will have to be paid to controlling outbreaks of the disease late in the season. This would not only keep the foliage healthy, incidentally preventing ascospore development,

but would also prevent infection of the bud-scales, and thus make control of the disease comparatively easy the following year.

Summary.

Bud-scales and tissue below the base of the scales of many varieties of apples were found infected with the fungus *Venturia inaequalis* in January and February, 1937. These centres of infection were responsible for the early outbreaks of apple scab and for the subsequent serious development of the disease on unsprayed trees at Glasnevin in 1937. They also account for the early appearance of the disease each year on varieties like Bramley's Seedling in the absence of both scabbed wood and dead leaves.

A distinct correlation existed between the severity of scab on the tree in 1936 and the percentage of diseased buds found in the following winter, and also between the number of diseased buds present during the dormant season and the outbreak and development of scab on the tree in the following May and June.

Infected bud-scales provided conidia from February until they were thrown off, and pustules below the scales were fructifying as late as 15th July; in both cases the maximum sporulation occurred during May and June.

Under the conditions of the experiment infection from ascospores was ruled out, and each tree provided its own source of infection early in the season of 1937.

Very little spread of the disease occurred from tree to tree.

The absence of infected buds was of such paramount importance in 1937 in eliminating early attacks of the disease on the foliage, that its effect throughout the whole season approached in most cases that of three or four sprays.

Spraying with Bordeaux mixture in 1936, though it injured the foliage and russeted the fruit, practically eliminated scab from the trees, and no infected bud-scales were found on trees so treated. Spraying with lime-sulphur was not so good, especially in controlling the late development of scab in 1936, and as a result some infected buds occurred.

The effects of good scab control extends to the year following spray treatment.

Thorough spraying in 1937 controlled the disease effectively even when over 40 per cent. of the blossom buds had parasitized bud-scales.

Infection of the bud-scales may occur at any time during the growing season, those infected early in the summer fructify freely the same year, and those infected later develop mycelial cushions which produce conidia in the following spring.

Observations on scabbed one-year-old wood showed that the pustules may liberate conidia until the end of July, and they may be responsible for late outbreaks of scab.

ACKNOWLEDGMENTS.

This investigation was carried out under the direction of Professor P. A. Murphy, Division of Plant Pathology, Albert Agricultural College, Glasnevin, to whom I am indebted for his interest and criticism throughout the progress of the work. The observations on ascospore development in 1926 are also due to Professor Murphy. Thanks are also tendered to Mr. G. O. Sherrard, University Lecturer in Horticulture, not only for placing the experimental trees under my control, but likewise for giving every facility in carrying out the spraying programme.

EXPLANATION OF PLATE 19.

Fig. 1.—Photomicrograph showing cross-section of bud of Cox's Orange Pippin in February, 1937. Two well developed scab pustules are shown on bud-scales at A and A′ and a third occurred at B, but at the latter point the fungal cushion has fallen out of the section. (× 42.)

Fig. 2.—Photomicrograph showing cross-section of base of bud of Bramley's Seedling on 3rd March, 1937, and a well developed mycelial cushion with the torn cells of the host cortex along the top of the fungal tissue. The fungus is spreading to the right and invading a bud-scale. (× 80.)

Fig. 3.—Photomicrograph showing cross-section of scab pustule fructifying at base of bud-scale of Bramley's Seedling, 19th April, 1937. The entire black mass on top of the section is composed of densely packed conidiophores and conidia. (× 80.)

Fig. 4.—Photomicrograph showing infected bud-scale of Bramley's Seedling and development of two separate pustules which are about to coalesce underneath the outer tissue of the scale, taken 3rd March, 1937. The mycelial cushions have been slightly shrunken from the overlying host tissue by dilute carbolic acid. (× 50.)

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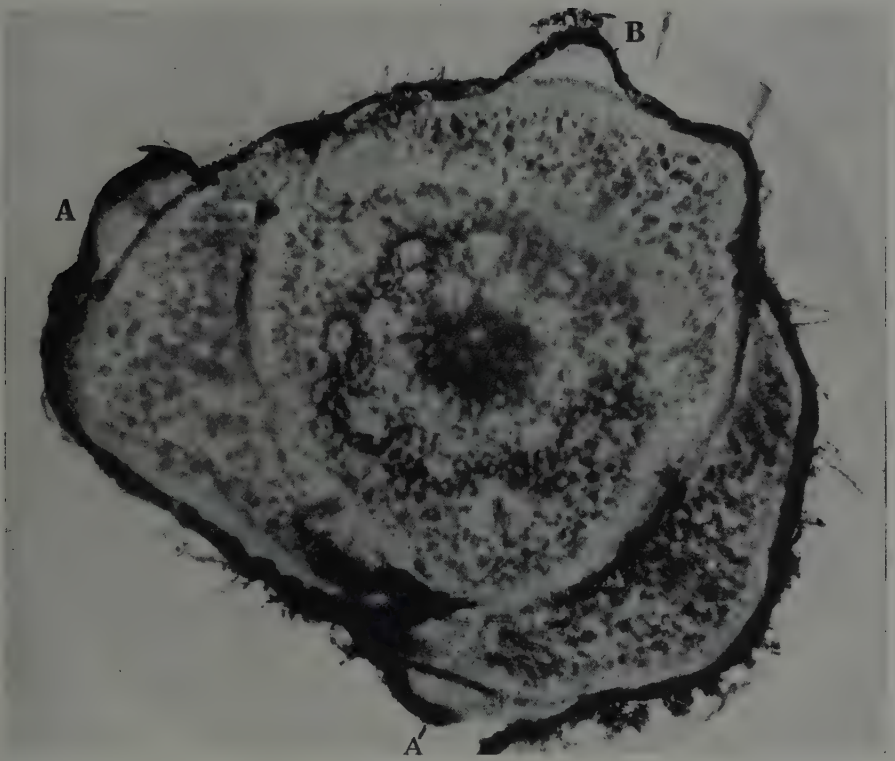

FIG. 1.

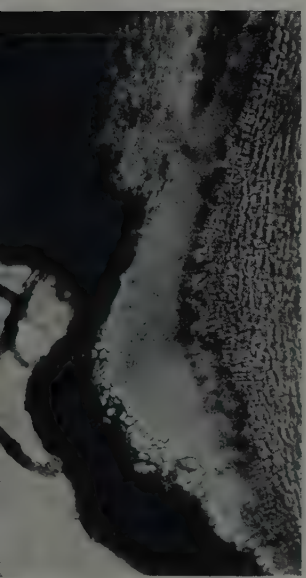

FIG. 2.

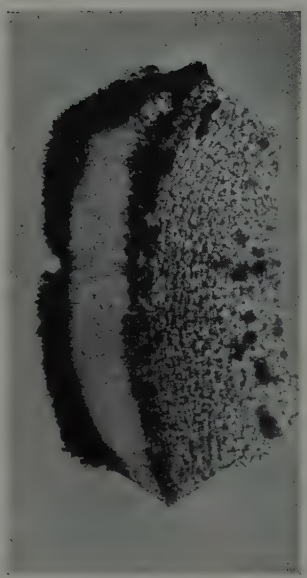

FIG. 3.

FIG. 4.

No. 58.

ANNUAL CHANGES IN THE OSMOTIC VALUE OF SOME ARCTIC AND TEMPERATE PLANTS.

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It is well established that most plants have a higher concentration of solutes in their sap during the winter than during the summer, and this change has been associated with the seasonal variation in temperature of their environment (for investigations of yearly changes in osmotic pressure see among others Dixon and Atkins, 2, 3, 4; Gail, 5; Lewis and Tuttle, 10; Steiner, 15; Thren, 16; Ursprung and Blum, 17; Walter, 20, and Winkler, 22). It has also been shown that the cell sap concentration, or osmotic value, rises more than would be expected, due to the actual decrease of water-content of the cells during periods of water shortage; this increase may be shown to exist in cells not short of water at the time of the determination of the osmotic value (see among others Braun-Blanquet and Walter, 1; Iljin *et al.*, 6; Korstian, 9; Simonis, 14; Ursprung and Blum, 18, and Walter and Walter, 21). In spite of this volume of work on the effect of water shortage there has been very little study of the possible mechanism of this regulation of cell sap concentration by water shortage, as most of the work has had an ecological basis. The effects of both temperature and drought are presumably reversible.

The writers had the opportunity[1] of spending the year 1935–36 in Kangerdlugssuak, East Greenland, lat. 68° 30′ N., and a series of determinations were made throughout the year of the osmotic value of cells of various plants as part of a more general physiological ecological study of the arctic vegetation. By way of comparison determinations have been made in Dublin during 1936–37 of the osmotic value of three of the species used in Kangerdlugssuak which also grow in Dublin.

The osmotic values of the plants were determined by a plasmolysis method.

[1] This work was carried out on the British East Greenland Expedition, 1935–36, under the leadership of L. R. Wager. (For a general account of the expedition and the work carried out see Wager (19).) The thanks of H. G. W. are due to the Governing Board of Trinity College, Dublin, for granting a year's leave of absence to go on the expedition.

Sections of the tissue, or stripped portions of the epidermis, were placed in a series of cane sugar solutions differing from each other by 0·1 Molar, and the degree of plasmolysis of the cells noted after about half an hour. The osmotic value of the tissue has been taken as being the concentration at which no visible plasmolysis of the cells was produced. In all cases the next higher concentration of sugar did produce slight plasmolysis in most of the cells.

The osmotic values determined in this way are probably slightly too high because of the contraction of the cell wall when the turgor pressure of the cells is removed, but this amount is probably nearly constant for any given tissue, and so in comparing a series of results it may be neglected. The accuracy worked to was not high, but sufficient in material which in exceptional cases varied by as much as 0·3 M. in osmotic value in parallel samples, and commonly by as much as 0·1 M.

The material was used straight after collection in the summer months, and in the winter was sometimes collected and left for about a half an hour to thaw out, and sometimes allowed to warm up gradually in a room at about −1° C. overnight and then brought into the laboratory and used. In either case no difference in the plasmolysis value was observed. The determinations in Dublin have been made within a few hours of collection in all cases.

In neither the Greenland nor the Dublin sets of determinations has the material been collected at a uniform time of day, and no allowance for variations from that source has been made. Neglect of the daily variation in osmotic value is justified in the present work, as the magnitude is generally not larger than 0·05–0·08 M. sugar (see Jimbo, 7, 8, and Ursprung and Blum, 18), and so falls well within the variability of parallel samples of material.

Notes on the Plants used.

The following general notes about the material may be given. Plants were snow-covered from October 6th until April or May, and the exact time of thawing out for each plant is shown on the graphs of the data.

The condition of the cells requires comment. The chloroplasts in all the material examined during the winter were disintegrated, and the whole of the protoplast was of a more or less uniform green colour. This effect disappeared again in the spring. A similar condition has been described for other plants by Lewis and Tuttle (10) and Zacharova (23).

It is possible that this is some effect of rapid warming, but the writers made many experiments with different plants, warming up the material very slowly and in different ways, but were unable to obtain whole chloroplasts in the winter material, and Raisanzew (13) describes changes, decrease in size, and clumping together, of the chloroplasts of certain plants with the onset of cold weather which were shown to be fairly rapidly reversible by keeping the plants in a warm room. These changes are probably a preliminary stage of the disintegration observed by the writers, Lewis and Tuttle, and Zacharova, and so it is reasonable to assume that this disintegration is not a result of the thawing out.

In the winter the protoplasm of many cells appeared to be alveolar, and the outline of the protoplasm was in consequence crinkled. This was especially pronounced in the cells of *Saxifraga oppositifolia*, and the same effect, though in a milder degree, was noted in the Dublin material of this species during the cold period in March.

Vaccinium uligonosum, subsp. *microphyllum*, Lge. (Fig. 1).—The plants were growing on a well-watered south-facing slope, and whilst it is impossible

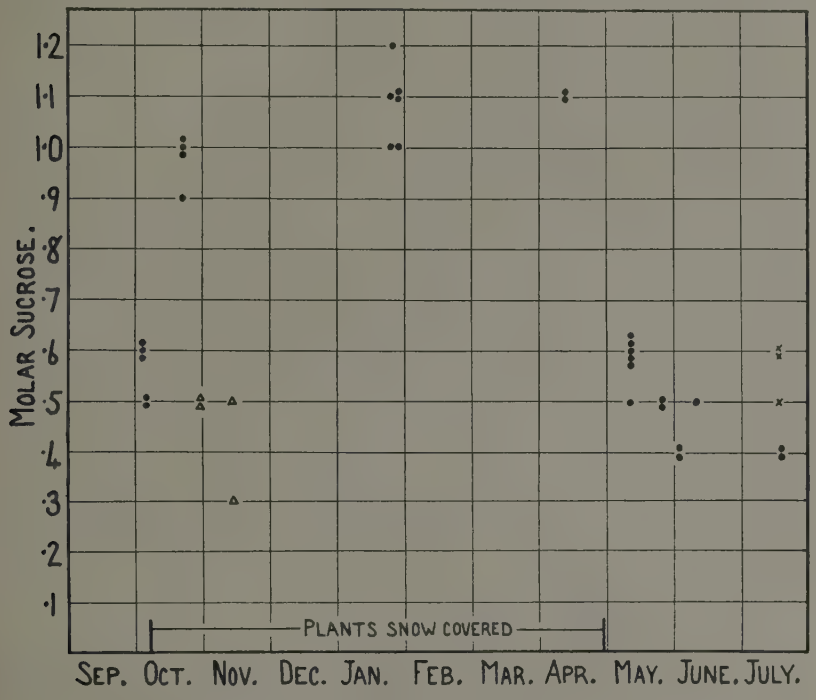

FIG. 1. Osmotic values of cortical cells of twigs of *Vaccinium uligonosum*. △ Material collected on October 17 and left in laboratory. × New season's growth.

to say that all the determinations were made on the same plant, they were all made on twigs collected within a metre or two of the same place. The osmotic value of the cells of the inner cortex of one-year-old twigs was determined in sections. A separate value on the graph means that a separate twig was used.

The twigs used for the determination of October 17th were left in the laboratory, and they were in leaf by the beginning of November and in flower by the end of the month; osmotic values of these twigs were determined on October 28th and November 11th, and are shown on the graph by triangles.

The rate of fall of the osmotic values corresponds very well with that observed in the spring, and is evidence that, at any rate in this species, the changes in the osmotic value occur as a result of temperature changes.

Empetrum nigrum (Fig. 2).—There were two plants investigated in Greenland, of which plant A was growing in a slightly more exposed situation than

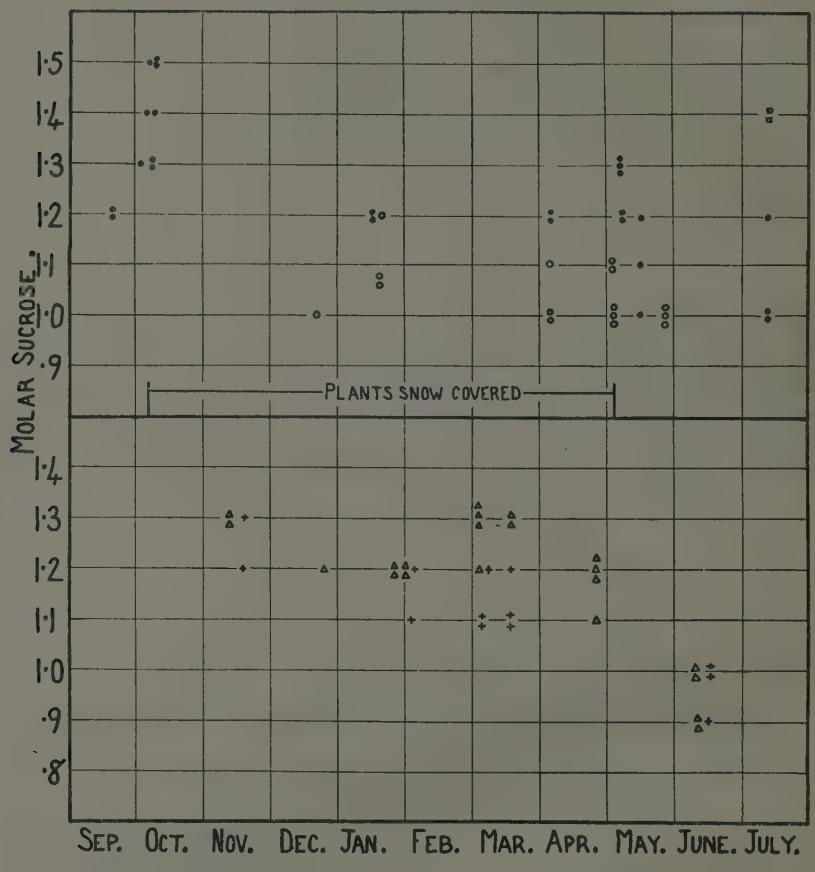

FIG. 2.—Osmotic values of palisade cells of leaves of *Empetrum nigrum*. Upper graph, Greenland plants. ● Plant A. Plant o B. Lower graph, Irish plants. △ Plant growing in Trinity College Botanic Gardens. + Plant growing at 1,100 feet in Co. Dublin.

plant B. It thawed out later in the spring and started growth later. Sections of the leaves were investigated, and the osmotic values of the various tissues noted: the values plotted in the graph are those of the palisade cells near to the mid rib. The sixth to the tenth leaf from the apex was used except in the case of

the determinations on the new season leaves (squares). These latter are higher, corresponding more to the values of the previous September.

Plotted in the lower part of the graph are the values obtained in Dublin from two plants—one growing in the Trinity College Botanic Gardens, and the other growing in the Dublin Mountains at about 1,100 feet above sea level.

Viscaria alpina, L., G. Don (Fig. 3).—All the determinations were made on the leaves of three plants growing close to each other on a south-facing and rather dry ledge. The osmotic values of the epidermis and of the mesophyll cells were recorded. These values were the same or else differed by 0·2 M., or

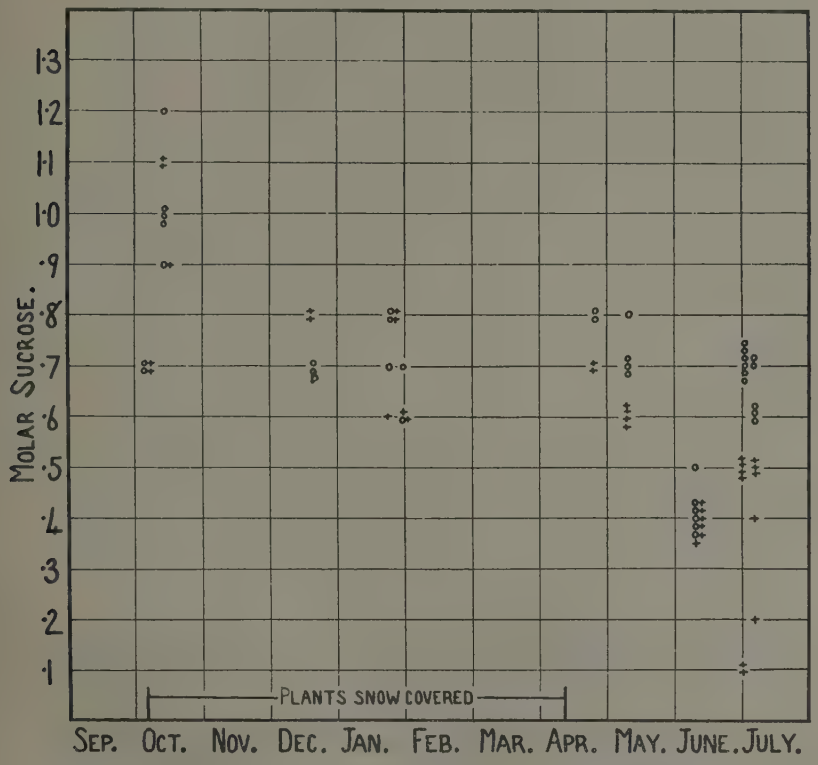

FIG. 3.—Osmotic values of leaf cells of *Viscaria alpina.* o Epidermal cells. + Mesophyll cells.

less, during the year until the determinations in July, when one of the plants gave the following pairs of values:—

Epidermis	...	0·7	0·7	0·7	0·7 M. sucrose.
Mesophyll	...	0·2	0·3	0·1	0·1 M. sucrose.

The other plants at this time showed no more than the usual variation. Such a large difference in the osmotic values of parts of the same tissue is very unusual, and this was the only case to be noted in the present work. The only visible external difference in the two plants was that the leaves of the exceptional plant contained no red pigment, as is usual in this species, and so may have been unhealthy.

Salix herbacea, L. (Fig. 4).—Three plants, all growing within about 30 metres of each other and under similar conditions, were used for the Green-

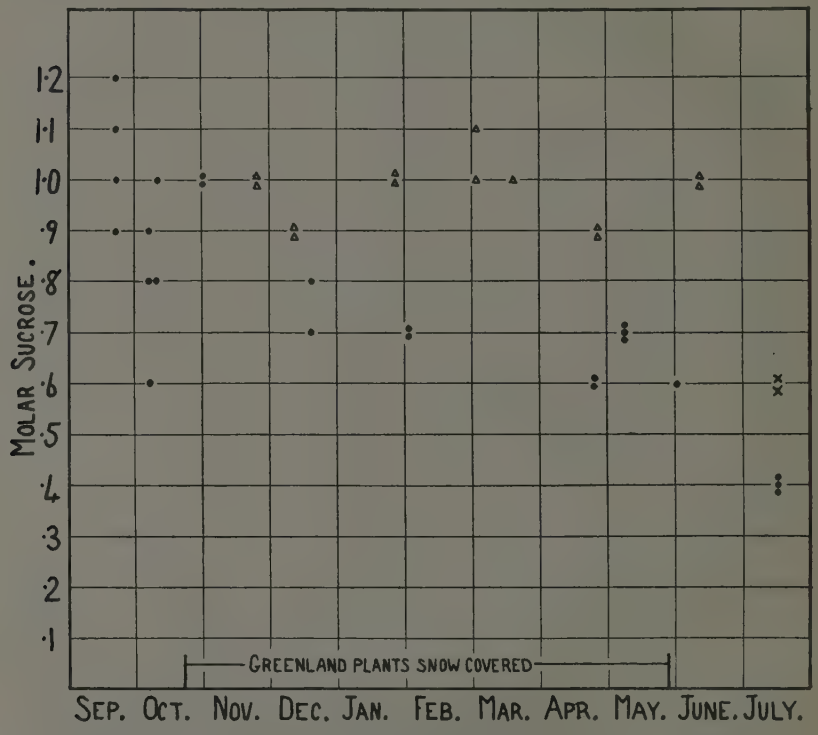

FIG. 4.—Osmotic values of cortical cells of twigs of *Salix herbacea*. ● Greenland, 1935–36. × New season's growth in Greenland. △ Dublin, 1936–37; a plant transplanted from Greenland in 1932.

land determinations, which were made on the inner cortical cells of one-year-old twigs. The new growth had a higher osmotic value, and is shown by the crosses.

The determinations made in Dublin were on some plants brought back from Greenland in 1932, and since grown in the Trinity College Botanic Gardens. These plants are younger than those used in Greenland, but one-year-old twigs were used, as in the Greenland plants.

Salix glauca, L. (Fig. 5).—Two plants were used growing about a hundred metres apart, and at no time did they show significantly different osmotic values. The single determination in September is probably quite significant. The new spring growth, when tested in July, had a higher osmotic value than the old, and is shown on the graph by crosses. The osmotic value of the cells of the inner cortical region of one-year-old twigs was determined.

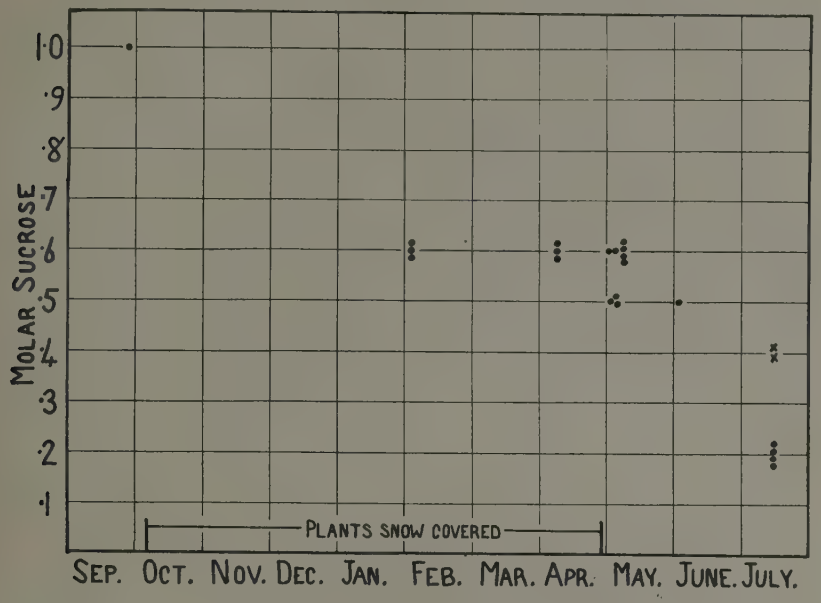

FIG. 5.—Osmotic values of cortical cells of twigs of *Salix glauca* ● Old twigs. × New season's growth.

Saxifraga oppositifolia, L. (Fig. 6).—Osmotic values were determined on about four different plants which grew in slightly different positions. The most interesting point is that the three values determined on March 6th were from a plant that had been continuously exposed throughout the winter, whereas the equal values of March 12th were from a plant that had been under snow all the winter. There would have been a difference in temperature of 8–12° C. on the average between the two plants for the previous three months, and yet they show no difference in osmotic value. This suggests that, at any rate in this plant, there is a limit of adaptability to low temperatures.

Two individuals were used for the osmotic value determinations in Dublin—the one a plant that had been growing for many years in the Trinity College Botanic Gardens, and the other a plant transplanted from Greenland in 1932 and now growing healthily in the same gardens. Both plants show the same

osmotic value throughout the year. The slight rise at the beginning of March is probably a response to colder weather during the previous two weeks.

Cerastium. cerastoides, L., N. L. Britton (= *C. trigynum,* L.) (Fig. 7).—All determinations were made on the mesophyll cells of leaves of the same plant. No determinations could be made during the winter, as the plant got so snowed up that it could not be dug out.

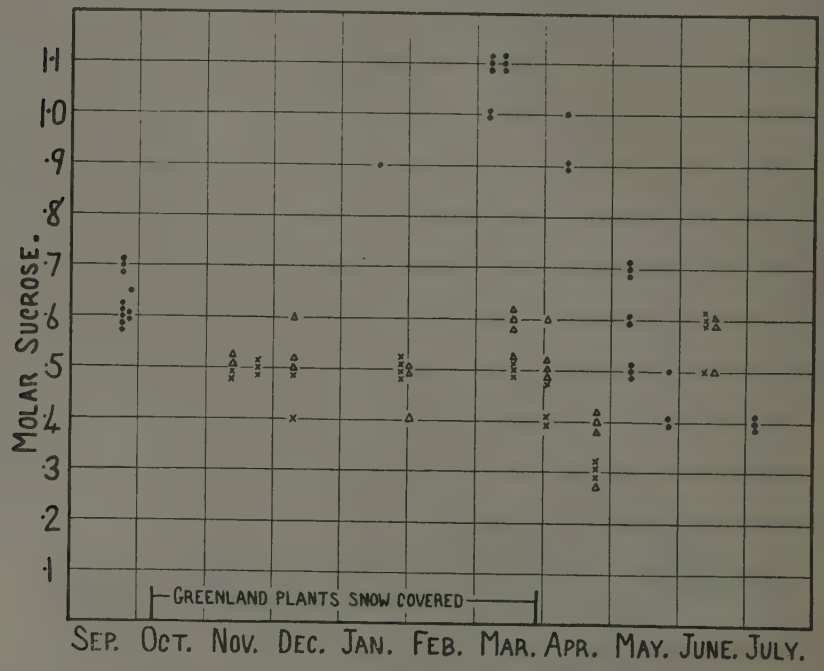

FIG. 6.—Osmotic values of epidermis of leaves of *Saxifraga oppositifolia.* ● Greenland, 1935-36. × Dublin, a Dublin plant. △ Dublin, a plant transplanted from Greenland in 1932.

Cassiope hypnoides, L., Don.—The following determinations of the osmotic value of the mesophyll cells of the leaves of *Cassiope hypnoides* were made:—

Oct. 29, 1·0 M.	Feb. 5, 0·8 M.	April 24, 0·8 M.	July 18, 0·6 M.
1·2	0·8	0·8	0·5
1·2	0·8		

RESULTS.

The data obtained have been presented in the form of graphs, plotting the osmotic value, expressed as Molar sucrose against the time of year that the determination was made. Each separate run on a separate leaf or twig is

plotted as a single point, and when several fall on the same value a series of points has been plotted in a vertical line grouped round the appropriate ordinate. For instance, in Fig. 1 on October 2nd three runs gave a value of 0·6 M. and two of 0·5 M. for the osmotic value of the twigs.

The main features shown by these graphs of the yearly changes in the osmotic value may be summarised as follows:—

1. The osmotic value of all the plants is higher during the winter than during the summer, and the change in value takes place rapidly during the spring.

2. The osmotic value of *Viscaria, Empetrum,* and *Vaccinium* rises very rapidly in the late autumn.

3. The osmotic value of *Salix glauca, Salix herbacea, Empetrum, Viscaria,* and *Cassiope* falls during the winter whilst the plants are under the snow.

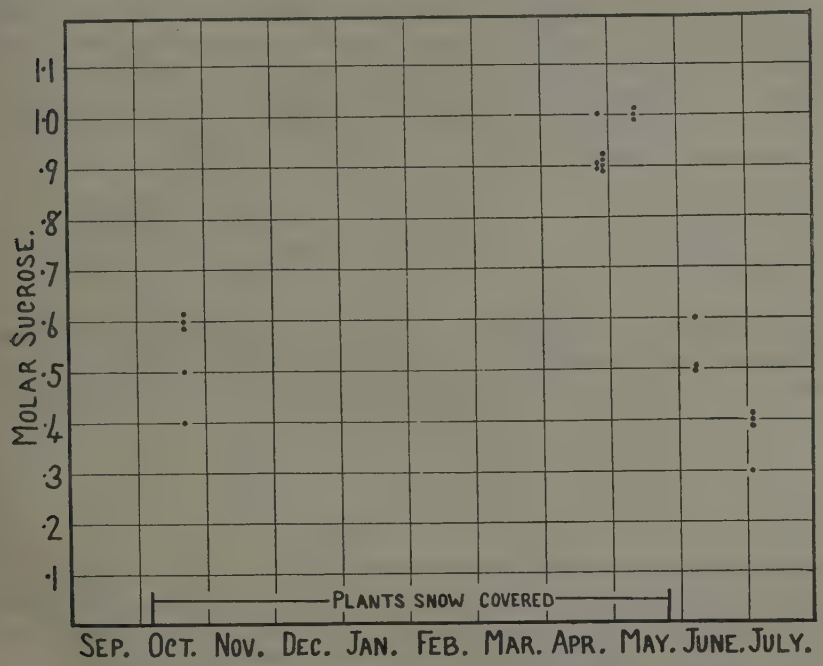

FIG. 7.—Osmotic values of mesophyll cells of leaves of *Cerastium cerastoides.*

CLIMATE.

The causes of the changes in osmotic pressure of plant cells probably lie mainly in the climate of their environment, so an account of the salient features of the climate of Kangerdlugssuak in 1935–36 will be given (see also G. Manley, 11, 12).

In September the temperature was falling rapidly, as shown by the following figures :—

Date.	Sept. 15–21.	Sept. 21–28.	Sept. 28–Oct. 5.
Weekly average minimum temperature ...	−1·7° C.	−3·6° C.	−5·5° C.
Weekly average temperature	1·0° C.	−0·4° C.	−3·0° C.

There was no precipitation of any sort during September, and the first snow-fall of the winter occurred on October 6th. Thereafter snow accumulated till it reached a depth of about 2 metres by the end of March. The temperature at the surface of the ground just after the first snow-fall was about −1° C., and then during the autumn slowly fell to a value of about −7° C., at which it remained steady for the whole of January, February, and March. The first thaw was at the beginning of April, and subsequently the temperature never fell much below freezing point at the ground level. The ground mostly cleared of snow during May and the first half of June, and growth of the plants commenced as soon as they appeared, as the air temperature was by then fairly high. The average temperatures and total precipitation for the summer are shown below.

	June.	July.	Aug. till the 17th.
Average temperature ...	3·7° C.	6·4° C.	5·8° C.
Precipitation	4·5	0·45	4·8 cm.

The average relative humidity of the air during the summer months was 73 per cent., but large deviations occurred for short periods. Water shortage of the plants, as judged by wilting or by the appearance of the soil, was rare in spite of the small amount of rain.

Discussion of Results.

One of the most obvious features of the results is their dissimilarity, in spite of the very similar environmental conditions of all the plants. In all cases there is a fall in the osmotic value in the spring, and so it is to be presumed that there is a slow late summer or autumn rise in value, although this is not shown due to the absence of determinations during this period.

The sharp rise in the late autumn shown by *Viscaria, Empetrum,* and *Vaccinium* is probably to be explained as a combination of several factors. The temperature was rapidly falling, but one aspect of this fall was to make the ground "physiologically dry," and the rise in the osmotic value is probably a result of a combination of falling temperature and increasing drought. A further effect of low temperature is to increase greatly the viscosity of both

water and protoplasm, and this will result in a slowing of the rate of transpiration, and so will accentuate the water shortage of this period of the year.

One of the most interesting features of the results is the fact that five plants, *Salix glauca, Salix herbacea, Empetrum, Viscaria,* and *Cassiope,* show a marked fall in osmotic value during the winter whilst buried under snow. The temperature probably rose slightly as a result of the snow cover, but would very soon fall again as the winter advanced (cf. section on climate), and so direct temperature change is probably not the cause of the decrease. Alteration of the cell-wall elasticity would affect the results, but it is difficult to believe that a sufficiently large change could occur. If there were water crystallised out in either the cell-walls or in the intercellular spaces it would probably indirectly result in this fall of osmotic value. On the other hand, the writers had no reason to believe that such ice crystals were present.

The most probable cause of this fall in osmotic value would seem to be alteration in the water balance of the cells. As stated in the introduction, no mechanism has been put forward to explain the effect of soil moisture on osmotic value, but it is to be presumed that the decrease in cell volume, and so concentration of solutes in the cell consequent on partial desiccation, must affect the starch-sugar balance in the cells. If some such mechanism as this is concerned in the regulation of osmotic value by water-shortage the change in value should depend only on the cell-volume, and be independent of the cause of this change; and, further, it should be readily reversible both by increase of water in the soil and by increase of atmospheric humidity. In September the air was relatively dry, and the soil cold, so water loss continued and water uptake from the soil was restricted; in consequence the plants were short of water, whereas under the snow the air is continuously saturated with water vapour. This water shortage was clearly shown by some plants, particularly by *Empetrum* and *Viscaria,* among the plants examined. In late autumn the protoplasts of these plants, by examination in oil, were seen to be much shrunken, and large spaces were present between them and the cell walls; but later, after the plants had been snow-covered for some time, the protoplasts had expanded, and again filled the cells. In this case the snow causes the slow saturation of the cells with water, and with this decrease in water deficit a return to a lower osmotic value, and so the observed downward drift of osmotic value under the snow may be explained. In those cases where the late autumn fall in osmotic value does not take place it is to be supposed that either those plants are more influenced by temperature changes than humidity, or that the plants did not suffer from water shortage in the autumn.

The fall in the osmotic values which all these plants show in the spring may be satisfactorily explained as being partly a temperature effect and perhaps partly due to a utilisation of reserves in growth, which is a cause suggested by Dixon and Atkins (2, 3, 4), Gail (5), and Korstian (9). It should be noted that in most cases the fall commenced before the snow cover had thawed off the plants, and during the period when the temperature of the surface of the ground

was at about freezing point (cf. section on climate). At this time the plants showed no visible signs of growth, and this suggests that the temperature change at any rate initiates the fall.

Comparison of the osmotic values of the Greenland and Dublin plants of the same species throughout the year shows the rather interesting but negative result that there is no consistent difference, as *Saxifraga oppositifolia* has a much lower osmotic value in temperate regions, *Empetrum nigrum* an equal, and *Salix herbacea* a rather significantly higher one. The explanation of this is probably that the group of factors controlling the level of the osmotic value of a plant is complex, and that different factors are of preponderating influence in different species. No analysis of these factors could be made with the data at present available. The only observation which may be relevant to this question is that the Salix growing in Dublin was a relatively young plant, only 10–15 years old, whereas the Greenland Salix plants were 30–40 years old at least.

The osmotic values of the plant of *Empetrum* growing at sea level and at an altitude of about 1,100 feet are not significantly different, except for the readings in March, and when those readings were taken the hill plant had been under snow for about three weeks. This suggests that a snow cover in temperate as well as in arctic regions tends to reduce the osmotic value.

The rise in the osmotic value of the Dublin plants of *Saxifraga oppositifolia* in June may be compared with that found by Dixon and Atkins (4), Gail (5), and Korstian (9), who find that as growth starts in the spring the osmotic value commences to fall, and does so till about the middle of the growing season, thereafter rising again fairly steadily. This has been partially associated with the increasing amount of osmotically active material in the plant, both organic and inorganic, but it would still appear to be uncertain whether the increasing water shortage as the summer proceeds is not the more important factor.

Summary.

1. The osmotic values of some seven arctic plants have been determined by a plasmolytic method at intervals throughout the year.

2. In the case of three of these species a similar series of observations have been made in Dublin.

3. Marked changes in the osmotic value occur at different seasons of the year, and these have been related to environmental factors as far as possible.

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No. 59.

THE pH OF CONIFER LEAVES IN RELATION TO SYSTEMATY.

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In spite of the importance of the conifers in forestry and as units of the world's vegetation our knowledge of many aspects of their physiology is still decidedly fragmentary. In this paper some facts are presented concerning the pH values of the cell-sap in different members of the group. It is hoped to deal later with an analysis of the nature of the buffering.

Earlier work in this laboratory (Doyle and Clinch, 9) claimed that the cell sap of the leaves of the large families of the Coniferales had a characteristic pH, and also that the pH of the leaves of a number of species did not vary much throughout the year. This investigation was only incidental to other work which was being carried on, and, as the method used was a relatively simple one, it seemed desirable to check these observations by a more accurate method and to extend the range of the investigation.

METHODS.

The estimations were made on expressed sap. Two methods of obtaining sap were used. In method A, 5 g. of fresh leaves were ground up in a mortar with 20 c.c. of boiled distilled water, and filtered through boiled dried muslin. The pH of this liquid, compared with the pH of expressed sap obtained by method B, always checked to within a couple of millivolts (0·05 pH). This method was fairly rapid, and, since it was shown to be dependable, was convenient for use; but very early in the survey it became apparent that it could not be used for all species. Many conifers contain considerable quantities of water-imbibing colloids, either in their saps or as some part of their cellular framework. If a few grams of the leaves of, say, *Cryptomeria, Araucaria araucana, Thuyopsis,* or *Sequoia gigantea,* are ground with water the mixture will set to a slimy mass incapable of being filtered or used with a hydrogen electrode. These colloidal substances do not seem to be confined to any particular family, and, as already shown by Doyle and Clinch (9), and confirmed by this work, they do not seem to be related in any way to the pH of the sap among the species examined.

Method B was the standard method of Dixon and Atkins (6). The tissues were frozen in liquid air, allowed to thaw, and pressed out in a vice between two iron plates, surfaced with silver foil. This method was very satisfactory, except for the labour involved in squeezing out tough leaves like those of *Picea,* and large enough quantities of clear straw-coloured saps could be obtained. As the hydration of the colloids in the saps obtained by method B was much less than by method A they were quite suitable for use with the hydrogen electrode.

The objection to either of these expressed sap methods is that the sap obtained consists of the probably differently constituted saps of the individual leaf tissues. Nevertheless, the technical difficulties in the use of, and the uncertain accuracy of the results obtained by, micro-electrode methods in the investigation of the pH of cells *in situ* are well known (17). Small's Range Indicator Method hardly seems to be applicable to tissues such as conifer leaves, which contain much chlorophyll.

The pH values were determined by the gas-chain method, using a saturated KCl calomel electrode. The hydrogen electrode used for most of the work, the rather simple type described by Cole (5), was found to be very satisfactory. The more elaborate rocking electrode of Clark was also used, and found far to surpass the others in the rapidity with which it reached equilibrium and in its economy of hydrogen. The potentiometer used was the Cambridge Instrument Company's portable potentiometer, type B, which read to 0·5 of a millivolt (in practice it was read to the nearest millivolt), and the whole apparatus was kept in a constant temperature chamber at 20° C., thus avoiding hysteresis effects in the calomel ½-cell, and simplifying the calculation of results. Standardization of the electrode was carried out according to the instructions of Clark (4).

The electrometric measurements of the pH of the saps were also checked by a B.D.H. capillator. The chlorophyll present in method A sap generally settled down in about 20 min., giving a clear fluid, quite suitable for use. The capillator measurements almost invariably agreed with the electrometric measurements, e.g., if the capillator gave pH 4·0 to 4·2 the H-electrode gave pH 4·1 or some figure within this range.

The criteria suggested by Clark (4) were used as an index of the behaviour of the electrode, i.e., a constant potential rapidly reached which could be reproduced by other electrodes and re-attained after polarisation, and an absence of drift in the galvanometer needle. Sluggish electrodes sometimes caused inconvenience, but when familiar with the apparatus it was generally easy to tell when it was behaving properly.

The H-electrode has been used for many biological fluids, but certain objections have been raised against its use—(*a*) that it displaces volatile constituents of the investigated fluid, such as CO_2, from a bicarbonate system; (*b*) that the reduction taking place at the platinum black may give rise to reduced substances with different dissociation constants, and thus alter the

ionic equilibrium in the fluid; (*c*) that oxidation reduction potentials will affect the electrode.

It is hoped to deal with the first point in a later communication on the buffer systems in conifers. For the present, it suffices to say that no trace of a bicarbonate system has been found even in the leaves with higher pH. Clark (4) has already considered the second point, but a few tests were made in this connection by estimating the pH with the capillator before and after using the H-electrode. No colorimetric change was ever detected. The substances responsible for oxidation potentials in these conifer saps are probably tannins, but other investigators using the H-electrode do not report any difficulties with plant saps or extracts. Thus Hoagland (13) used it for work on the sap of Barley, while Wilson and Kern (19), using it with tan liquors, found that no trouble was experienced up to pH 9.

No difficulty was found in conifer saps in reaching equilibrium with a hydrogen electrode; the pH obtained checked colorimetrically; and, if a titration of the sap with acid or alkali was followed with the H-electrode and the capillator, both agreed quite well on both the acid and alkaline sides. Consequently it is felt that the pH values obtained are quite reliable.

The quinhydrone electrode was considered and tried at an early stage, using a larger foil electrode instead of the wire electrode. This gave good results on buffer mixtures, but when tried on the conifer saps it gave results which were much higher than the other two methods, which, as already stated, checked closely. These errors of the quinhydrone electrode are due to the presence of sugar + phosphates and tannins (17). Indeed, quinhydrone is used as the B.D.H. spot-test reagent for phosphates, and, as will be shown in a later communication, there is an appreciable concentration of phosphate in the leaf sap of at least some conifers. Since the potential of the quinone-hydroquinone system at any given pH depends on the ratio of one to the other, any substances which react with either result in false potentials. Thus, La Mer and Parsons (15) say that error arises through hydroquinone or quinone being converted into the other by some active oxidising or reducing agent present in the sap, or by their unequal conversion into other bodies, and Doyle and Clinch (8) have remarked on the abundance of reducing substances in these leaf saps. As well as the phosphate + sugar, and the "reduction oxidation error," this method also suffers from a tannin error (17), due to condensation of the quinone or hydroquinone with the tannins. This error, up to 0·7 pH, can be corrected by successive dilutions. Since dilution reduces the tannin error, the observed pH decreases to a point, and then commences to increase, and since the hydrion concentration cannot be increased by dilution, the lowest pH is taken as the correct one.

A number of tests were made along these lines. The pH of pure sap obtained by method B was determined with the H-electrode and the quinhydrone electrode. Similar samples of the same sap were then freed from tannin by treatment with hide powder for 24 hours, and the pH again determined. Successive dilutions of the two saps were also tested with the quinhydrone

electrode. The following figures for *Larix decidua* will show the general nature of the results:—

pH Values of *Larix* Sap.

	H. electrode.	QH. electrode.	Successive dilutions.
Pure sap . .	3·63	4·0	4·0 – 3·75 – 3·65 – 3·8
Sap treated with hide powder .	3·7	3·8	3·8 – 3·7 – 3·7 – 3·9

Young leaves did not show these differences, presumably due to a lesser tannin content. Incidentally, when quinhydrone is added to these saps it is quite obvious that it is reacting with them, as there is a brownish precipitate. The dilution method with these saps, which may be very highly buffered, is, however, tedious and uncertain, as it takes considerable dilution, 10–15 times, to cause a decrease in pH, and at every addition of water it is necessary to add a little more quinhydrone to keep the liquid saturated. Obviously titration would be next to impossible; and, though the quinhydrone electrode may be useful for clean saps, for conifer leaf juice it suffers from too many errors.

The only other paper available on the pH of conifer leaf sap was that by Goldsmith and Smith (12) on the physico-chemical properties of the sap of leaves of Engelman spruce. They seem, however, to have experienced difficulty in determining the initial acidity, and it is not easy to decide on the correctness of some of their data. They state that the initial acidities are difficult to determine with the hydrogen electrode, as they found it practically impossible to get a platinum electrode to reach equilibrium, a difficulty which disappeared on the addition of small amounts of HCl or NaOH. No explanation of this effect is offered, and the use of the quinhydrone electrode is suggested as a solution of the problem, but not apparently put into practice.

While recognising that their initial pH values are not accurate, they present nevertheless the seasonal changes in pH of leaf sap from trees at four different stations in a table which shows extraordinary variations. From the station "Shelter Rock" there is a drop of pH 0·96 from March to June, followed by a sudden rise of pH 1·28 in July in the older leaves. The young leaves are pH 2·2 in June, and rise pH 1·7 to pH 3·9 in one month. From the station designated "Half Way" the older leaves, pH 3·9 in March, drop to pH 2·64 in June, followed by a sudden rise to pH 4·2 in July. The young leaves from the four stations range from 3·8 to 3·86 in October, a reasonable figure for spruce; but in December one station is pH 3·66, another pH 2·42, while the third and fourth have fallen to the extremely low figures of pH 1·96 and pH 1·55. Such changes in pH in a strongly buffered sap, as the spruce has been found to be, would seemingly necessitate the production of very large amounts of acid.

Although *Picea Engelmanni* was not studied in our work, none of the other species examined showed such extremes of variation. Seasonal changes were

small but fairly regular. No difficulty was experienced in getting the hydrogen electrode to give a steady E.M.F., which it would hold indefinitely, even with such saps as that of *Torreya californica*, containing an unpleasant oily substance which might be expected to poison the electrode. It is possible that Goldsmith and Smith's difficulties were caused by incorrect deposition of platinum black or by impure hydrogen, or a faulty electrode with minute cracks or a scratched surface. It is thus very doubtful how far any of their figures, particularly the records of the large rapid changes, can be accepted.

A considerable part of the material was obtained from the Botanic Gardens, Dublin, through the usual courtesy of the Keeper, J. W. Besant, to whom we wish to express our sincere thanks. Such material was brought direct to the laboratory, and used within an hour or two of gathering. Similar fresh material was also supplied by Mr. H. M. Fitzpatrick, of the Forestry Department, from Delgany, Co. Wicklow. The rest of the material was obtained from the gardens and estates of Powerscourt, Kilmacurragh, and Mount Usher, all in Co. Wicklow, and from Rostrevor Gardens, Co. Down. We also wish here to thank the owners and gardeners of these estates, as well as the Curator of the Botanic Gardens, Kew, from whom *Actinostrobus*, *Glyptostrobus*, and adult *Widdringtonia* were obtained. Although some delay was attached to the material from Powerscourt it could usually be dealt with on the day of collection; but, owing to the distance, collections from the other estates could not be examined till the following day, and were kept in water overnight, during which period they changed only very slightly or not at all.

The plant names used, with the exception of a few readily recognized ones, are those given by Pilger (*Gymnospermae* in Engler and Prantl, 13, 1926).

RESULTS.

The pH values thus obtained with the H-electrode, checked with the capillator, may be presented under the following heads:—(*a*) Individual and regional variations; (*b*) seasonal variations and variations due to age; (*c*) general survey in relation to systematy. The data under (*a*) and (*b*) are still incomplete, and only refer to very few species, but are probably sufficient to give some idea of what the extremes of variation might be expected to be, information obviously necessary if any value is to be attached to the data given under (*c*). The species examined under this latter heading are more numerous, and include representatives of all genera except about eight. From most of the larger genera a number of species were also examined, selected from the different generic sections.

A.—*Individual and regional variations.* Little difference is shown among leaves of the same age on a single individual. Thus, leaves of *Tsuga heterophylla* at a 5-feet level at different parts round the tree showed a pH variation from 3·75 to 3·83, at 12 feet a variation from 3·75 to 3·93, and at 18 feet 3·96 to 4·1. This apparent increase with height has not yet been

sufficiently examined to determine how universal it may be. Leaves taken from twigs reasonably near together, on the same large lateral branch, for example, had practically identical pH values, which remained constant from day to day when examined over a short period. Such material can reasonably be used for the examination of seasonal change or change with age. Regional or habitat variations were examined in *Taxus baccata, Cupressus Lawsoniana, Picea excelsa,* and *Pinus silvestris.* Occasional observations are available on other species. Seven individuals of *Taxus* showed a variation from 5·35 to 5·6. Seven specimens of the cypress showed a variation from 5·05 or 5·1 to 5·25. These differences are no greater than those shown by a single tree. Nor could any obvious relations be seen between these differences and habitat variation. Thus, in the case of four specimens of the cypress growing in similar soil, the collections were taken respectively from branches (*a*) well illuminated all the year, (*b*) heavily shaded at all times by an *Abies* growing very close and overhanging, (*c*) shaded in summer by a sycamore, and (*d*) in medium shade all the year. The corresponding pH values were 5·1, 5·05, 5·2, 5·15. Similar remarks apply to *Taxus.*

The data referring to *Pinus silvestris* and *Picea excelsa* are given in greater detail in Tables 1 and 2.

TABLE 1.

Habitat Variation in *Pinus silvestris*—April, 1937.

	Capillator.	H. electrode.	Quinhydrone.
Powerscourt, No. 1 . .	3·8 – 4·0	4·0	4·3
„ „ No. 2 . .	4·0 – 4·2	4·05	4·45
„ „ No. 3 . .	4·0 – 4·2	4·1	4·5
Scalp, No. 1 . .		4·1	
Powerscourt, No. 4 . .		4·1	
„ „ No. 5 . .	4·0 – 4·2	4·2	4·4
Delgany		4·25	
Glencree, No. 1, 1,200 ft. .	4·2	4·3	4·6
„ „ No. 2, 1,000 ft. .	4·4	4·35	4·7
Pine Forest, 800 ft. . .		4·35	
Albert College, Glasnevin .	4·4 – 4·6	4·5	4·9
Glencree, No. 3, 750 ft. .	4·6	4·5	4·9
Scalp, No. 2 . . .		4·5	
Powerscourt, No. 6 . .	4·6	4·6	4·95

TABLE 2.

Habitat Variation in *Picea excelsa*—April, 1937.

	Altitude.	H. electrode.
Pine Forest, No. 1 . .	1,000 ft.	3·65
„ „ No. 2 . .		3·75
Glencree, No. 1 . .	1,000 ft.	3·9
„ No. 2 . .		3·95
Pine Forest, No. 3 . .		3·95
Delgany	250 ft.	3·95
Glencree, No. 3 . .		4·0
Scalp, No. 1 . .	500 ft.	4·05
„ No. 2 . .		4.15
Pine Forest, No. 4 . .		4·2

Table 1 shows the pH values given by the capillator used as a check on the hydrogen electrode, and the values obtained by the quinhydrone electrode. The latter always gives results higher by about 0·35 pH.

As in the case of *Cupressus* and *Taxus*, there is also here no indication of any relation between pH value and habitat variation in soil, altitude, or light. The specimen of pine, for example, growing practically at its altitudinal maximum of 1,200 ft. at Glencree on a poor acid peaty granitic soil, shows the same value as the one at Delgany, growing on a good arable soil, at an elevation of only 250 ft. For that reason the figures are entered in the order of pH magnitude. The tables show a maximum variation of approximately 0·6 pH, which is greater than in the case of the less acid *Taxus* and *Cupressus* types. This is to be expected, since even if the actual acidity variation were the same in the 3·5–4·5 range as in the 5–6 range, the pH variation should be less in the higher range. But in these examples the variation in the more acid range is relatively greater than the actual variation found in the less acid range. This may be merely due to the fact that fewer examples were examined in this range, or because species like the spruce and pine, especially the pine, are rich in sub-species or varieties with perhaps corresponding pH differences; and no systematic examination has been yet given to the sub-specific rank of the groves and isolated individuals of pine and spruce planted in Ireland by private owners. The numbers available are obviously not sufficient for any statistical examination. In *Picea*, however, the variation rather resembles a normal type, and 7 out of 10 lie between 3·7 and 4·1. The figures for *Pinus* almost suggest a bi-modal variation, as if two races were under examination; and undoubtedly some forms were different from others. Thus, the plant No. 1 from the Scalp had quite definitely smaller tougher leaves, with a lower sap content than most of the

others. This variation has, however, not yet been examined systematically. In general, the range 4·1 to 4·5 covers 11 out of 14. It might also be remarked that these variation estimations were made in April. Reference to Fig. 1 in the succeeding section will show that at this period in the pine there seems to be a seasonal fluctuation rising to the summer level. Greater variations are, therefore, to be expected at this period. The figures given in the later systematic survey were all taken between November and January, when conditions are much more stable, the values generally a little lower, and, although this is uncertain, the general variation possibly less.

Temporarily, it may be concluded that the pH variation between leaves from different individuals is not much greater than that from different parts of the same tree. As an approximation, it may be suggested that that variation may be $\pm 0{\cdot}3$ from the average, in some cases possibly a great deal less.

B.—*Variation with age and seasonal Variation.* At any season the pH of young leaves is less than that of similar older leaves from the same branch. A few of the values obtained are given in Table 3. In this table the young leaves are the current leaves of the year, the old leaves are those of the previous year.

TABLE 3.

pH of Young and Old Leaves.

	June.		July.		Oct.–Nov.	
	Young.	Old.	Young.	Old.	Young.	Old.
Pinus excelsa . . .	3·7	4·4	3·9	4·5	3·9	4·5
Tsuga heterophylla . .	3·2	3·7	—	—	3·55	4·0
Sequoia sempervirens . .	—	—	3·6	3·7	3·7	4·0
Picea Breweriana . .	3·45	4·8	3·9	4·8	5·1	5·3
Podocarpus andinus . .	—	—	3·75	4·2	4·0	4·3
Taxus baccata . . .	4·7	5·3	4·9	5·4	5·3	5·5
Torreya californica . .	—	—	5·4	5·5	5·4	5·7

This increase in pH with age seems to be a well-known phenomenon in angiosperms. Bennet-Clark (3), Newby and Pearsall (16), Auber (quoted by Evans, 11), Small (17), and others, all refer to a lessening of free acid or an increase in pH in mature leaves. For conifers Goldsmith and Smith (12) had also recorded that the pH of young Engelmann spruce leaves in July was lower than that of older leaves. Since acidity as such will be considered in a later communication no further analysis need here be made of these figures, except to call special attention in connection with *Picea Breweriana* to the fact that although the pH of old leaves may be more than 5, yet that of the youngest

leaves is about 3·5. The interest of this will appear in the paragraphs dealing with the general systematic survey.

The nature of the change—i.e., whether gradual or fluctuating—in young leaves from their expansion in early summer till late autumn of the same year has not been properly followed out. The pH values given in the earlier paper by Doyle and Clinch (7) cover this period, but show no regularity in drift, except a fairly steady pH increase with age in *Larix*. This period should be examined with a more accurate method than the one used by them. Some data, however, are available on subsequent changes in a number of species. Starting in November or December, i.e., with a class of leaves already about six months old, this class was examined at intervals through the succeeding year to June or July. It has already been mentioned that leaves of similar age from the same branch show practically identical pH values, which do not change from day to day over a short period. The material, therefore, in each case was always taken from the same large branch from the same tree, and, to minimise diurnal fluctuations, if any, was always collected at about the same hour. The collections, made fortnightly or monthly, are perhaps too far apart to permit of general conclusions, but the results seem to show definite seasonal drifts. From among the results available eight are shown graphically in Figs. 1 and 2. These have been chosen from among those in which the collections were more regular, to illustrate the variety of seasonal fluctuation which may occur.

The changes shown in Fig. 1 by the two very different pines, *P. silvestris* and *P. Montezumae*, are surprisingly similar, and are paralleled by two other

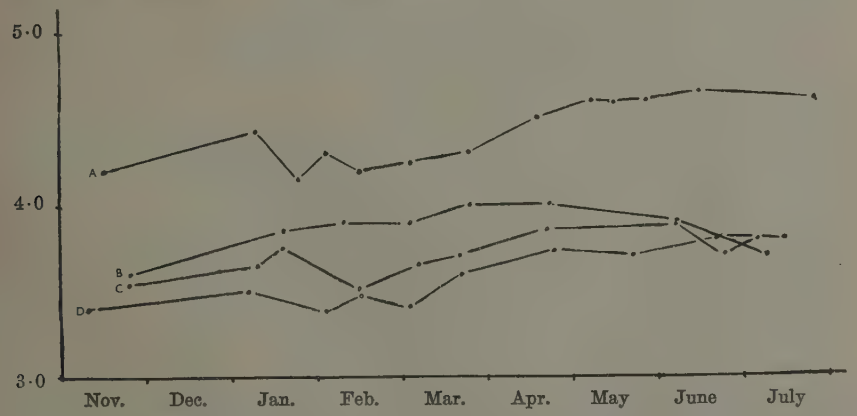


FIG. 1.—Seasonal pH variation in A, *Pinus silvestris*; B, *Sequoia sempervirens*; C, *Tsuga heterophylla*; D, *Pinus Montezumae*.

pines which were examined, *P. nigra* and *P. excelsa*. A rise in pH to mid-winter is followed by a lessened value, which fluctuates for a couple of months and then rises to a summer level. The *Tsuga* curve is rather similar, but shows

also a slight drop in early summer. This drop might be ignored here were it not that it appears in so many other cases. *Cedrus* and *Keteleeria* agreed closely with *Tsuga*. *Sequoia sempervirens*, on the other hand, shows no spring drop, but a constant rise, followed by a drop in late spring and early summer. It is of interest in this connection to remember that the redwood, in common with other forms native to a Mediterranean type of climate, such as *Cupressus macrocarpa* and *C. sempervirens*, does not show, at least under Irish conditions, a long winter break in growth. The lateral branches at least often show active growth in late autumn, and, if conditions are mild, well into winter, resuming growth again very early in spring. The curves in Fig. 2 show the changes in

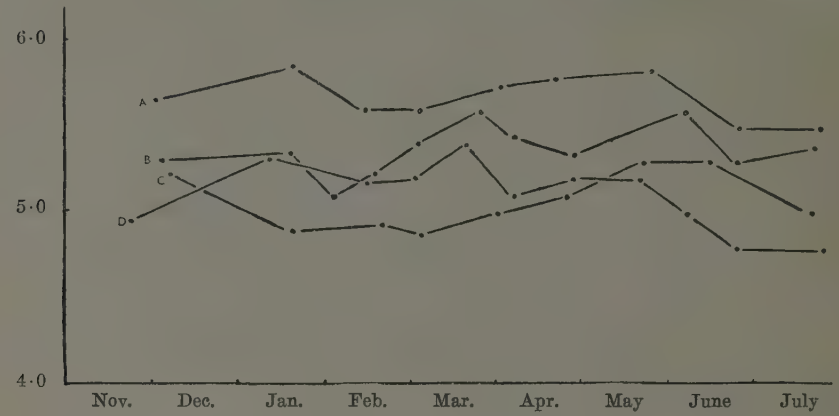


FIG. 2.—Seasonal pH variation in A, *Torreya californica*; B, *Taxus baccata*; C, *Podocarpus totara*; D, *Picea Breweriana*.

four less acid types. *Torreya*, while resembling the *Pinus-Tsuga* group in the earlier part of the record, shows a marked summer drop; and *Taxus*, although showing greater fluctuations, still displays the spring drop fairly clearly. The interesting spruce, *Picea Breweriana*, in which both the spring and summer drop can be seen, shows still greater fluctuations, which reflect perhaps the fact that it is less strongly buffered than most other conifers examined. In *Podocarpus totara* the spring fall is long maintained, rendering the summer rise of short duration before the second fall sets in.

It would be easy to lay too great emphasis on these results. The differences are small, and the possibilities of error perhaps considerable, but the parallelism shown by the abietinean forms, as well as the greater fluctuations shown by the less strongly buffered *Picea Breweriana*, seem to show that the changes are not merely sporadic. The drop in early spring, which is shown by all forms with resting buds, may be of definite interest, as this is the period in which the plants are preparing for the break in the winter rest. A similar decrease in pH associated with the inception of new growth in apple twigs has been noted by Abbott (1), and Goldsmith and Smith (12), although

their figures are perhaps not very reliable, also record rather similar seasonal changes in the sap of the Engelmann spruce. Although there may not be many accurate studies of pH changes throughout the year, yet that this period, preceding the new activity in spring, is a critical one has been shown by many workers. As far as conifers are concerned, Ishibe (14), for example, describes changes in the carbohydrate content of *Pinus* stems at this period, while Doyle and his co-workers (8, 10) record changes in starch and catalase in the leaves of many species. A paper by Stälfelt (18) on the variation in chlorophyll content in the common spruce and fir throughout the year is of special interest as some of the curves shown very closely resemble these pH curves at least between November and June.

Much further work is needed before these pH changes can be substantiated or explained. The nature and amount of available acid can vary with the relative activity of the metabolic processes. The available base, whether absorbed from the soil or set free internally, can also vary. Few data are available concerning such variations. Zacharova (20) reports tremendous variations at relatively short intervals in sun leaves of *Picea excelsa,* variations so considerable that they should be reflected in marked pH changes. No such changes were apparent in any estimations here made on the spruce. It is hoped to examine the whole problem more adequately in the near future. In the meantime it seems safe to say that seasonal pH changes do occur in conifer leaves, and are related to the seasonal rhythm in their metabolism.

C.—*General Survey in relation to systematy.* More than 90 species were examined between mid-November and early January. The results are shown in Tables 4 and 5. These results are presented with considerable reserve. The pH values from exotic specimens in cultivation here may differ from those in their native habitat, although this is considered unlikely, except, perhaps, at the climatic extremes of their natural range. From the two preceding sections it is also clear that variation exists between different individuals of the same species. A proper table could only be prepared with such a full knowledge of the variation range in each species as would permit an estimation of a proper average. This is obviously at present impossible, as some of the forms are very rare in cultivation, only three small plants of *Microcachrys,* for example, existing in the whole of Ireland, and only one known specimen of *Libocedrus uvifera.* It has already been suggested that a variation range of $\pm 0{\cdot}3$ from the average may be assumed as an approximate maximum. If the figures presented be taken as average figures, this 0·3 change would actually affect the main results comparatively little. In a few cases, perhaps, a greater allowance might be necessary, resulting in the appearance of a few more aberrant types like *Picea Breweriana,* but there is little reason to expect that these cases would be anything but rare. Leaves of most of the species examined seasonally show a fair degree of stability in the November and December of their first year's development, and as this period was most convenient for the wider survey it was carried out during these months, although a few could not be covered till

early January. Most of the material was thus of comparable age. Recognising, however, the difficulties, and emphasizing the provisional nature of the results, nevertheless the figures so far available present many features of interest. No endeavour is made in the following rapid commentary to include references to systematic work. Many points are well known to students of the conifers, others are expressions of personal opinion.

The earlier results of Doyle and Clinch (9) claimed that the great conifer groups had each a distinctive pH range. The Abietineae and Taxodineae (except *Sciadopitys*, pH 5) all lay within a 3·4–3·8 range, the Cupressineae and Taxaceae within 5–5·6, while the only member of the Araucarineae examined, *Araucaria araucana*, lay close to the more acid forms with pH 3·9. While many of these results agree closely with those presented in this paper, the inclusion of a larger number of genera and the more accurate methods of measurement, show that, while in a rough general way the original claim holds, there are large and important exceptions. The original claim was related to an older classification. When, however, the newer figures are related with more modern ideas of classification it is found that many of these apparent exceptions are just the results which hold the greatest interest.

Examination of these tables shows that the genera and species are grouped in the principal families as now commonly considered. The larger families are subdivided partly on a basis of family sections, and partly for the purpose of clearer presentation. The species are arranged in a semi-graphical manner. In the vertical direction the species are arranged spatially in relation to their pH. Thus, the pH of any form can be approximately read off by comparing its vertical position with the pH values marked on the left. The vertical distance between any two is thus also a measure of the difference in pH between them. In general, pH 4·5 can be taken as a rough critical line, and the main bulk of genera can be divided into two groups, one more acid with a $pH < 4{\cdot}5$, and one less acid with a $pH > 4{\cdot}5$. In cases where a number of varying readings were available from different trees the average is recorded. Table 4 contains the families Pinaceae (old Abietineae), Taxaceae (old Taxineae), and Araucariaceae (old Araucarineae). The Pinaceae show a solid block of more acid genera and species. Although in other families generic or tribal differences in pH range seem to exist there is no such specialization here, the variation within the larger genera overlapping the smaller; nor is any indication given of the clearly existing sub-division of the family into the *Pinus* group, the *Picea-Larix-Pseudotsuga* group, and the group formed by the other five genera. This is in agreement with the fact that the *Pinaceae* is a very compact family, the subgrouping referred to being definitely of secondary importance. The narrow range within which the five closely related genera, *Abies*, *Cedrus*, *Pseudolarix*, *Tsuga*, and *Keteleeria*, are included is remarkable, although it would probably be extended if more species of *Abies* were examined. One outstanding exception in the genus *Picea* is shown by *Picea Breweriana*, the mature leaves of which show a $pH > 5$, in one branch up to 5·35. This is not

only a rather rare spruce in cultivation, but in its natural distribution the rarest of all the spruces, being confined to a few isolated localities in the Siskiyou Mountains of N.W. California and S.W. Oregon, although quite successful in these localities. It is apparently adapted to a special grouping of environmental factors which may be related to a peculiar metabolism. It is definitely more poorly buffered than most other conifers, even the less acid ones in other families, and shows among other peculiarities a very large content of minute crystals in its sap. The young leaves, on the other hand, show a pH value not only well within the Pinaceae range, but actually low for that family. It is clearly a very special member of the Pinaceae, and one which might well repay more detailed study.

The Taxaceae all belong to the less acid group, *Cephalotaxus Fortunei* being more acid than the others. In this group, however, the generic pH values do not overlap. This may be an expression of the recognized differences which exist between the three genera, *Taxus, Torreya,* and *Cephalotaxus,* a difference which is by some emphasized by the removal of *Cephalotaxus* as the representative of a special family, the Cephalotaxaceae. The difference between the average pH of the species of *Cephalotaxus* and of *Torreya,* or of *Torreya* and *Taxus* combined supports this idea. It is of further interest that *C. pedunculata* is so much closer to *C. drupacea,* of which it is commonly considered a variety, than either is to the much more distinct species, *C. Fortunei.*

The Araucariaceae, on the other hand, belong to the more acid group, the Kauri, *Agathis australis,* being the most acid of all the conifers examined. Here, too, the generic difference between *Agathis* and *Araucaria* is, perhaps, indicated by the pH difference. It is of further interest that species of *Araucaria* differ also from those of *Agathis* in having sap which is extraordinarily rich in hydrophilous colloids; so much so in *Araucaria araucana* that it yields, even with liquid air, a jelly-like sap, with which the H-electrode could not be used. The figure given is a capillator one only.

These three families, with the exception of *Picea Breweriana,* are clearly in agreement with the general claim that the conifer groups show each a distinctive pH range. In the case of the other families, shown in Table 5, the results do not quite so obviously fall into line, but appear to be all the more interesting. The large family of the Cupressaceae includes many genera which should probably be separated into distinct families, but appear so interlinked in many characters that a proper expression of their relationship is at present difficult. In the first column appear the true cupressoid genera which are quite definitely closely related. They form a compact block on the less acid side of pH 4·5 except the one extreme reading from *Cupressus macrocarpa,* probably the lowest possible limit on the acid side. The largest genus *Cupressus* includes all the others, as might be expected; while, as far as the figures go, the *Chamaecyparis* section seems to show a less acid tendency than the *Eu-cupressus* section. It is interesting also that *Cupressus Leylandii,* a hybrid between *C. nootkatensis* and *C. macrocarpa,* shows a pH value intermediate between that of the parents.

TABLE 4.

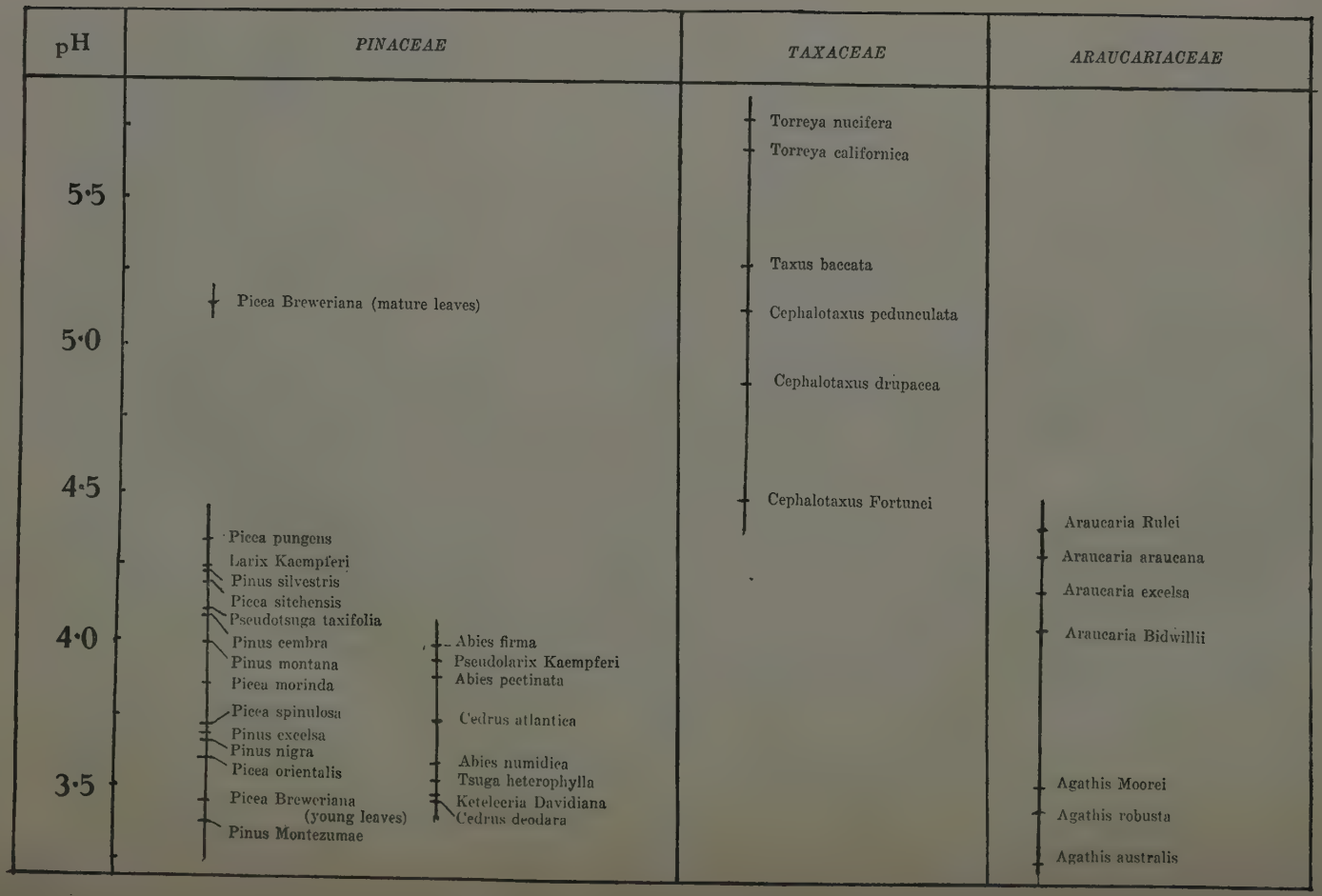
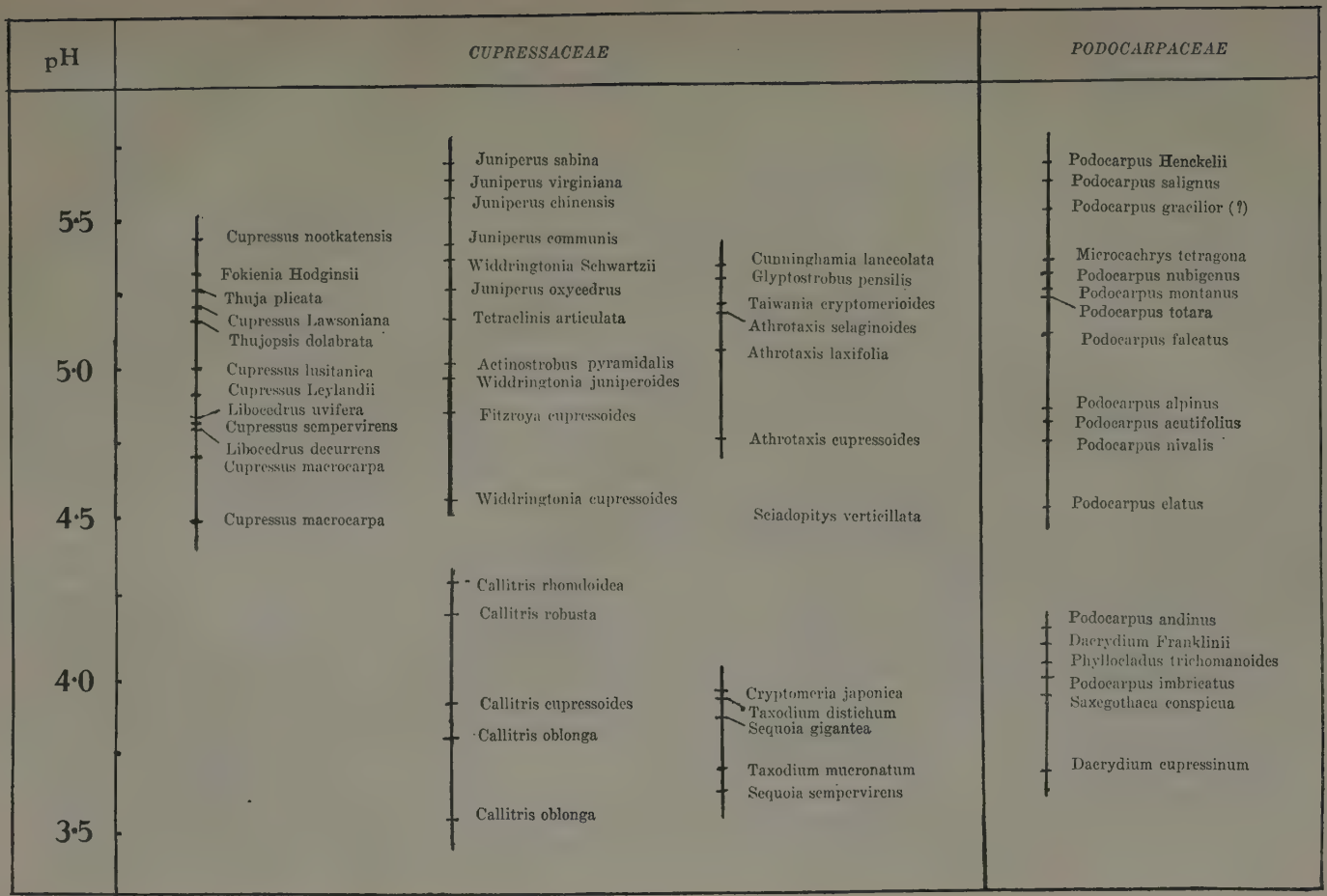

pH
PINACEAE
TAXACEAE
ARAUCARIACEAE
5·5
5·0
4·5
4·0
3·5
Picea Breweriana (mature leaves)
Picea pungens
Larix Kaempferi
Pinus silvestris
Picea sitchensis
Pseudotsuga taxifolia
Pinus cembra
Pinus montana
Picea morinda
Picea spinulosa
Pinus excelsa
Pinus nigra
Picea orientalis
Picea Breweriana (young leaves)
Pinus Montezumae
Abies firma
Pseudolarix Kaempferi
Abies pectinata
Cedrus atlantica
Abies numidica
Tsuga heterophylla
Keteleeria Davidiana
Cedrus deodara
Torreya nucifera
Torreya californica
Taxus baccata
Cephalotaxus pedunculata
Cephalotaxus drupacea
Cephalotaxus Fortunei
Araucaria Rulei
Araucaria araucana
Araucaria excelsa
Araucaria Bidwillii
Agathis Moorei
Agathis robusta
Agathis australis

TABLE 5.


pH
CUPRESSACEAE
PODOCARPACEAE
5·5
5·0
4·5
4·0
3·5
Cupressus nootkatensis
Fokienia Hodginsii
Thuja plicata
Cupressus Lawsoniana
Thujopsis dolabrata
Cupressus lusitanica
Cupressus Leylandii
Libocedrus uvifera
Cupressus sempervirens
Libocedrus decurrens
Cupressus macrocarpa
Cupressus macrocarpa
Juniperus sabina
Juniperus virginiana
Juniperus chinensis
Juniperus communis
Widdringtonia Schwartzii
Juniperus oxycedrus
Tetraclinis articulata
Actinostrobus pyramidalis
Widdringtonia juniperoides
Fitzroya cupressoides
Widdringtonia cupressoides
Callitris rhomboidea
Callitris robusta
Callitris cupressoides
Callitris oblonga
Callitris oblonga
Cunninghamia lanceolata
Glyptostrobus pensilis
Taiwania cryptomerioides
Athrotaxis selaginoides
Athrotaxis laxifolia
Athrotaxis cupressoides
Sciadopitys verticillata
Cryptomeria japonica
Taxodium distichum
Sequoia gigantea
Taxodium mucronatum
Sequoia sempervirens
Podocarpus Henckelii
Podocarpus salignus
Podocarpus gracilior (?)
Microcachrys tetragona
Podocarpus nubigenus
Podocarpus montanus
Podocarpus totara
Podocarpus falcatus
Podocarpus alpinus
Podocarpus acutifolius
Podocarpus nivalis
Podocarpus elatus
Podocarpus andinus
Dacrydium Franklinii
Phyllocladus trichomanoides
Podocarpus imbricatus
Saxegothaea conspicua
Dacrydium cupressinum

The data in the second and third columns are more problematic. The second column includes in the first place *Juniperus,* placed here for convenience in recording only. In spite of the differences existing between them, its relationship to the other cupressoid forms is admittedly close. Its position in the table agrees with this. It overlaps the genus *Cupressus,* but shows a distinction in the development of species which are much less acid. The earlier figures of Doyle and Clinch also show the same tendency. It may be mentioned that in those junipers with dimorphic foliage no significant difference existed between the pH values of the juniperoid leaves and the cupressoid twigs. Now in many forms from different groups—*Cupressus, Thuja, Callitris, Dacrydium,* etc.—the twigs carry the adpressed cupressoid type of leaf, so that pH measurements can be made with convenience, not from leaves, but only from the small terminal twigs. Structurally, of course, these are mainly composed of leaf tissue, but it is important to find in those cases where awl-like leaves and cupressoid twigs are available that no difference exists between them. This second column mainly includes the callitrinean forms and a couple of genera of uncertain relationship. The callitrinean genera are *Widdringtonia, Actinostrobus,* and *Callitris.* Unfortunately many details are still lacking in the knowledge of the life-histories of these forms, but those who have worked on them consider that they represent a line from the cupressoid stock—a line in which *Widdringtonia* is nearest to the cupressoid ancestry, and *Callitris* is definitely a more specialized type. The coincidence of the range covered by the *Widdringtonia* species and by the true cupressoid genera, in contrast to the separation of the *Callitris* species, as a more acid group with pH values definitely $< 4{\cdot}5$, supports this view in an interesting way. On the other hand, the position of *Actinostrobus* seems exceptional. All the data available seem to point to a much closer relationship between *Actinostrobus* and *Callitris* than between either of these and *Widdringtonia*; in fact, they would appear as closely related to one another as the genera of the Pinaceae among themselves or as *Cupressus* to *Libocedrus.* If more species of *Callitris* were available this genus might prove one with a very wide range, like *Podocarpus* (in the fourth column), a range which might include *Actinostrobus* and overlap *Widdringtonia*; or *Actinostrobus* may be a definitely exceptional form, like *Picea Breweriana.* Only one small branch was available, sent by post from Kew Gardens, where a potted plant is growing. In spite of the fact that the specimen was kept for so long a period enclosed under abnormal conditions it is not considered probable that leaves examined fresh would yield a markedly different result, as tests made on similarly enclosed specimens of *Callitris* showed surprisingly little change from the fresh condition. It is, however, always possible that further work may show that from a morphological point of view its relationship to *Callitris* is not as close as it is at present supposed to be. *Tetraclinis* was formerly included in the callitrinean group on the basis of habit. Its life-history, however, has been shown to be quite different from the others, and to be cupressoid, in the strict sense, in most particulars. At present, from the

morphological point of view, it may be considered a cupressoid form which has developed the peculiar *Callitris* habit, a habit paralleled by so many *Casuarina* species in the angiosperms. If this be so it can obviously be transferred in the pH table, to the first column, and take there a natural place.

Fitzroya is a genus of very peculiar and uncertain affinity. It shows relationships to *Juniperus,* to the cupressoid complex, and probably also to *Widdringtonia.* Its position in the table, although agreeing with any of these, adds no emphasis in any particular direction, but supports the suggestion, made from morphological grounds, that it also is only distantly connected to *Callitris.* With the possible exception therefore of *Actinostrobus* and *Picea Breweriana,* the forms included so far seem to support the view that affinity shows itself in similarities of metabolism, of which one expression is a correspondence in pH range. This result is not so clear in the third column, and perhaps also in the fourth column of this second table.

The third column includes the taxodioid forms, commonly grouped as the Taxodineae or Taxodiaceae. Of these, *Sciadopitys* shows so many peculiarities of structure and development that it is frequently separated as the sole member of a special family. Its relationships are uncertain, showing as it does affinities with the Pinaceae, the true cupressoid conifers, the podocarps, and taxads. This uncertainty is reflected in its pH value, which falls on the 4·5 line, and gives no further suggestion as to its true position. Apart from *Sciadopitys,* the relationships among the main taxodioid forms are possibly more uncertain than within any other conifer group. Included here are a number of probably relict forms, often monotypic and endemic in certain areas; and, from considerations of structure and development, it is possible that there are grouped together here genera of widely varying affinities. Discussion of such affinities is out of the question in this paper, but the pH values seem to be an expression of this uncertainty. The demarcation into a more acid and a less acid group, and the separation in these different groups of forms such as *Sequoia* and *Athrotaxis,* which have frequently been united in a special tribe, are at once obvious. It is possible, on the other hand, that this separation is connected with real tribal distinctness. There are reasons for doubting the very close suggested affinity of *Sequoia* and *Athrotaxis,* for example; there are reasons to consider *Athrotaxis, Taiwania,* and *Cunninghamia* more closely allied to one another than any one is to *Sequoia* and *Taxodium*; but further knowledge is required before we can say whether in this taxodioid complex the metabolism, as indicated by pH values of the sap, is in any way related to true affinity, or whether such a relation, so strongly indicated elsewhere, here breaks down completely. It is of interest, however, to find that *Athrotaxis laxifolia,* a species remarkably intermediate between *A. selaginoides* and *A. cupressoides,* but closer to the former, takes up just this position in the table. The wide separation of the so-called swamp cypresses, *Taxodium* and *Glyptostrobus,* is particularly suggestive. Mainly on habit a close relation between these is commonly assumed. Habit, however, is a very illusive criterion of affinity, and perhaps

here the difference in the cones and in wood structure may really be of greater importance. But unfortunately nothing is known of the life-history of *Glyptostrobus* or of *Taiwania.* If that knowledge were available, coupled with much more information than we now possess concerning the older Mesozoic conifers, it might be possible to decide if the suggestive separation into the two groups on the basis of acidity is here anything more than merely an interesting fact.

The data concerning the Podocarpaceae are given in the fourth column. The genus *Podocarpus* shows a greater pH range than any other examined, a result to be expected, perhaps, from a consideration of the wide range occupied by the species both geographically and in relation to climate. The first species measured, *P. andinus, P. nubigenus,* and *P. salignus,* seemed to show a wide separation between the more acid *P. andinus* and the less acid *P. salignus,* comparable to the separation existing between, say, the Pinaceae and Taxaceae. This separation seemed of interest since *P. andinus* has been, and still frequently is, removed from *Podocarpus* as a special genus *Prumnopitys.* As many species as were available in reasonable condition were then examined to determine if such a separation really held in the genus. The table shows that one other species, *P. imbricatus,* can be coupled with *P. andinus,* most of the others falling closer to the less acid section. One species, *P. elatus,* was finally found partly bridging the gap between the two pH groups. If a still larger number of species were available a complete grade might be found between the pH 4 of *P. imbricatus* and the pH 5·6 of *P. salignus,* but there still exist indications of a separation of the genus into distinct pH groups. Although no sectional separation within the genus appears in the species of *Pinus* and *Picea,* such a separation is indicated in *Cupressus,* within which the *Chamaecyparis* species are less acid, and in *Juniperus,* where the species belonging to the section *Oxycedrus* do not overlap those belonging to the section *Sabina.* A similar situation may exist, to some extent at least, in *Podocarpus,* especially as the two acid species are *P. andinus,* already commented on, and *P. imbricatus,* a species which belongs to the special small section, *Dacrycarpus.* This section shows some resemblance to the genus *Dacrydium,* and it will be noted that the only two species of this genus available belong to the acid group, definitely marked off by structure and pH value from the main group of the other *Podocarpus* species. *Saxegothaea,* probably closely related to *Dacrydium,* takes its position with it in the table. *Phyllocladus* falls here also, but its relationships are too uncertain to warrant any comment. The position of *Microcachrys* is, however, remarkable. Morphologically it has been placed in the Podocarpaceae, with a probable relationship to *Saxegothaea,* but in the pH table it is widely separated. Recent work, however, has shown that, although a common type of gametophytic development is found, with variations, in all the other podocarps, yet the development in *Microcachrys* is very different, and resembles more closely that of *Cunninghamia,* or the typical cupressoid forms. The definite separation in pH value clearly reflects this developmental difference;

on the other hand, if it is removed from the podocarps and changed to the neighbourhood of *Cunninghamia* or the cupressoid group, this position would be quite in accordance with its pH value.

In general, therefore, it cannot be said that the conifer *families* are characterized by a distinct pH value. But, while emphasizing again the reservations made at the beginning of this section, it may at least be said that there seems to be in the conifers a strong tendency for natural affinity to be expressed in a similarity of metabolism which is related to a fairly narrow pH range. The data so far obtained seem sufficiently suggestive to be worth recording, but it is still necessary to survey much more completely some of the larger genera as far as material is available.

Reference has already been made to the frequent presence of hydrophilous colloids in the sap, and that in many cases these were present in considerable quantities, so that in working the saps could be roughly divided into sticky and non-sticky groups. Doyle and Clinch (7) found no relation between pentosan colloid and pH in the few forms examined by them. Although no quantitative examination has here been made, it is quite clear that their conclusion is correct in general; nor is the presence or absence of considerable quantities a tribal or even a generic characteristic. It is hardly necessary to compile a list, but a few examples may be noted and compared with the pH tables. Thus, the species of *Agathis* all yield a fluid mobile sap, while the sap from the *Araucaria* species is extraordinarily thick; *Sequoia sempervirens* and *Taxodium* contain little colloid, *Cryptomeria* and *Sequoia gigantea* contain much; *Podocarpus nubigenus* is poor, while *P. montanus* is rich; and so on. The presence or absence of these colloidal substances in the saps of closely related forms raises interesting questions as to their origin and functions, particularly in those species in which they are present in quantity. Some of these species are so rich that it would appear probable that their water relations must be controlled as much by colloidal inhibition as by simple osmotic forces. It is, perhaps, a matter of regret that, in the earlier pentosan work of Doyle and Clinch (7), the species, which had been chosen practically at random, were all species containing comparatively small amounts of such colloids.

SUMMARY.

(1) The methods of extracting sap and estimating its pH are described.

(2) The quinhydrone method is unsatisfactory for conifer sap.

(3) Individual and habitat variations are comparatively small, $\pm 0{\cdot}3$ pH from the average as an approximate maximum; in many, especially the less acid species, probably considerably less.

(4) No relation could be observed between these variations and habitat conditions of soil, altitude, or light.

(5) Seasonal changes are small but seem definitely related to seasonal rhythms in metabolism, particularly by a fall in pH in many species before the onset of spring activity.

(6) All the conifers examined lay within the pH range 3–6.

(7) In some cases complete families are characterized by a much narrower range—viz., Pinaceae, Araucariaceae, and Taxaceae.

(8) The large indefinite Cupressaceae as well as the Podocarpaceae are not so characterized, but the natural affinity of most of the smaller sections within them is expressed by definite pH groupings.

(9) There is in the conifers a very strong tendency for natural affinity to be expressed in similarities of metabolism which are reflected in the correspondence of the pH range of the related groups.

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